MEDICA

IVD CE

REF 10211-4 4 x 29 mL / 10 mL

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

INTENDED USE
The EasyRA HDL reagent is intended for the quantitative measurement of high density lipoprotein cholesterol in human serum or plasma, using the MEDICA EasyRA® Chemistry Analyzer in clinical laboratories. This HDL Cholesterol test has been certified by the Cholesterol Reference Method Laboratory Network (CRMLN).
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 presence of elevated amounts of high density lipoproteins (HDL) is associated with a decreased risk of coronary heart disease and it may also be a protective factor. The measurement of HDL cholesterol (HDL) helps in early diagnosis and treatment of lipid and lipoprotein metabolism disorders. Low HDL cholesterol levels have been strongly associated with increased risk of coronary heart and coronary artery disease.[2,3] Therefore, the determination of serum HDL-C is useful in identifying high-risk patients. 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] The reference method for HDL cholesterol is the Abell-Kendall analysis[5] However, since the reference method requires ultracentrifugation and precipitation and cannot be fully automated, homogeneous (direct) methods have been developed. This method is based on accelerating the reaction of cholesterol oxidase with non-HDL-unesterified cholesterol and dissolving the HDL selectively using a specific detergent (viz., "Accelerator Selective Detergent" method).

PRINCIPLE OF THE PROCEDURE
This direct assay method of measuring HDL cholesterol involves the removal of other non-HDL lipoproteins via selective reaction by Reagent 1. In the second step, the selective detergent in Reagent 2 solubilizes the HDL cholesterol specifically which then reacts with a chromagen to develop a color which can be read optically at 600 nm. The intensity of the color at the maximum absorbance at 600 nm is proportional to the concentration of HDL cholesterol in the sample.

REAGENTS

HDL REAGENT (R1):

Good's Buffer	
Cholesterol oxidase (E. Coli)	<1000U/L
Peroxidase (Horseradish)	<1300 ppg U/L
N, N-bis(4-sulphobutyl)-m-toluidine-disodium (DSBmT)	<1 mM
Accelerator	<1 mM
Preservative	<0.06%

HDL Reagent (R2):

Good's Buffer	
Cholesterol esterase (pseudomonas sp.)	<1500 U/L
4-Aminoantipyrine	<1 mM
Detergent	<2%
Restrainer	<0.15%
Preservative	<0.06%
Ascorbic acid Oxidase (Curcubita sp.)	<3000 U/L

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.

SPECIMEN COLLECTION AND STORAGE / STABILITY[5]
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 7 days at 2 – 8°C. If specimens need to be stored longer than 1 week before testing, they may be frozen at < -70°C for up to 3 months. Samples may be frozen only one time. Refer to CLSI H18-A for further instructions on specimen collection, handling and storage.

PROCEDURE

Materials Provided
Medica HDL Reagent Wedge, REF 10211

Additional materials required

Medica HDL Calibrator, REF 10653
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 HDL Calibrator, REF 10653, 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 HDL Cholesterol Calibrator was assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL).

Quality Control
It is recommended that 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. 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 HDL concentration from the ratio of the corrected unknown sample's absorbance to the corrected absorbance of the calibrator multiplied by the calibrator value.

$$\text{HDL (mg/dL)} = \frac{[(A_{U_{600}} - A_{U_{700}}) - (A_{RBlk_{600}} - A_{RBlk_{700}})] - [(A_{U_{600}} - A_{U_{700}})_{SBlk} - (A_{RBlk_{600}} - A_{RBlk_{700}})_{SBlk}] \times dF}{[(A_{C_{600}} - A_{C_{700}}) - (A_{RBlk_{600}} - A_{RBlk_{700}})] - [(A_{C_{600}} - A_{C_{700}})_{SBlk} - (A_{RBlk_{600}} - A_{RBlk_{700}})_{SBlk}] \times dF} \times \text{Cal Value}$$

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 HDL in the calibrator (mg/dL). 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 NCEP (National Cholesterol Education Program) defines a serum HDL-C concentration below 40 mg/dL as a significant independent risk factor for coronary heart disease. Similarly, concentrations above 60 mg/dL have been termed "negative risk" factors, emphasizing the protective effect of elevated HDL cholesterol.

Procedural Limitations (e.g. if sample is above assay range)
Avoid using heavily hemolyzed serum or plasma samples.

The EasyRA Chemistry Analyzer flags any result above 150 mg/dL as Linearity High “LH”. Do not schedule automatic rerun for results over the linear range for this test. The linear range cannot be extended above 150 mg/dL.

PERFORMANCE CHARACTERISTICS[7]

Reportable Range
The reportable range is 2 to150 mg/dL.

Inaccuracy / Correlation (CLSI, EP9-A2)

The following table lists the data obtained in a comparison of the Medica Reagent for HDL (y) on the EasyRA Analyzer to the performance of HDL 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	2 to 148 mg/dL
Slope	0.93	y Intercept	1.8
Correlation Coefficient	0.9976	Regression Equation	Y = 0.93*X + 1.8

**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 HDL 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

The accuracy of the HDL Cholesterol method was verified by comparison to the Designated Comparison Method (DCM) for HDL Cholesterol using 40 fresh samples at a CRLMN Laboratory. A Certificates of Traceability was issued for this method, which indicates the bias from the reference method ≤ 3% and a coefficient of variation (CV) ≤ 3%.

Imprecision (CLSI, EP5-A2)

Duplicate measurements of each of three 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 %
33.2	0.65	1.96
64.6	0.96	1.49
39.6	0.80	2.02

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
33.2	0.84	2.52
64.6	1.41	1.92
39.6	1.20	3.10

Linearity (CLSI, EP6-A)

Linear from 2 to 150 mg/dL, based on the linear regression Y = 1.026*X + 1.741. The linear range cannot be extended above 150 mg/dL .

Interfering Substances (CLSI, EP7-A)

Less than 10% change in the value was classified as “no significant interference.”

No significant interference was found in levels of up to 500 mg/dL of hemoglobin.
No significant interference was found in levels up to 30 mg/dL of bilirubin.
No significant interference was found in levels up to 1000 mg/dL of triglycerides (using Intralipid*).

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.[8,9]

REFERENCES

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3. Romm PA., et al., *Relation of Serum Lipoprotein Cholesterol Levels to Presence and Severity of Angiographic Coronary Artery Disease*. Am. J. Cardiol. (1991) 67: 479-483.
4. Special Communication, Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), JAMA Vol 285:19, 2486-2497 (2001).
5. Warnick G.; Russell;, Wood, Peter D., National Cholesterol Education Program Recommendations for Measurement of High-Density Lipoprotein Cholesterol: Executive Summary, Clin Chem, 41:10, 1427-1433 (1995).
6. Gordon T., et al., *High Density Lipoprotein as a Protective Factor against Coronary Heart Disease: The Framingham Study.* Am J. Med. (1977) 62: 707-714.
7. Data on file at Medica.
8. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
9. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EasyRA Assay Parameters (HDL)

Primary Wavelength (nm)	600
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
Linearity	2 to 150 mg/dL

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