MEDICA

IVD CE

REF 10204-4 4 x 39 mL

CHOLESTEROL (CHOL)
Wedges each contain a usable volume of 39 mL of reagent.

INTENDED USE
The EasyRA CHOL reagent is intended for the quantitative measurement of cholesterol in human serum or plasma, using the MEDICA EasyRA® Chemistry Analyzer. This 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
Cholesterol is a steroid which is synthesized primarily in the liver and intestinal wall. Roughly 25% of total cholesterol is of dietary origin. The serum cholesterol level is the result of lipid metabolism and is affected by heredity, diet, and organ functions (e.g. liver, kidney, thyroid and endocrine). High levels are associated with increased risk of arteriosclerosis and cardiac risk.[1]

PRINCIPLE OF THE PROCEDURE
The enzymatic Trinder endpoint reaction, based on the work of Allain et al.[2] is as follows:

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta 4\text{-cholesterone} + 4H_20_2$$

$$2H_20_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye} + 4\ H_20$$

The absorbance of the resulting quinoneimine dye is measured at 520 nm with 600 nm as a blanking wavelength.

REAGENT

Pipes buffer, pH 6.7	50 mmol/L
Phenol	24 mmol/L
Sodium cholate	5 mmol/L
4-Aminoantipyrine	0.5 mmol/L
Cholesterol esterase	≥ 180 U/L
Cholesterol oxidase	≥ 200 U/L
Peroxidase	≥ 1000 U/L

PRECAUTIONS
1. Good laboratory safety practices should be followed when handling any laboratory reagent. (*CLSI,* GP17-A2).
2. The reagent contains less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Material 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 and protected from light. 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
Clear unhemolyzed serum or plasma should be used. Plasma samples must be collected using lithium heparin as anticoagulant. Centrifuge and remove the serum or plasma as soon as possible after collection. Specimens should be drawn from fasting patients. Serum Cholesterol is stable for 5-7 days at 2 – 8°C, for 3 months at -20°C and for years at -70°C.[3]

PROCEDURE

Materials Provided
Medica CHOL Reagent Wedge, REF 10204

Additional materials required

Medica EasyCal Chemistry, REF 10651
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 neck of the wedge for foam after removing the cap and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Medica EasyCal Chemistry, REF 10651, 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.

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 cholesterol concentration from the ratio of the absorbance of the unknown sample to the absorbance of the calibrator, multiplied by the calibrator value.

$$\text{CHOL (mg/dL)} = \frac{[(A_U - A_{Blk})_{520} - (A_U - A_{Blk})_{600}]}{[(A_C - A_{Blk})_{520} - (A_C - A_{Blk})_{600}]} \times \text{CalValue}$$

Where A_U and A_C are the absorbance values of the unknown and the calibrator, respectively; A_{Blk} is the absorbance of the reagent blank; and "Cal Value" is the concentration of cholesterol in the calibrator.

Expected Values
Risk groups have been studied to classify the acceptable concentrations of total cholesterol in serum.[4] The following risk groups have been identified:

Risk Classification	Total Cholesterol
Desirable	< 200 mg/dl (5.18 mmol/L)
Borderline High	200 –240 mg/dl (5.18-6.19 mmol/L)
High	≥ 240 mg/dl (6.22mmol/L)

At least two measurements of cholesterol on separate occasions should be performed before a medical decision is made. A single point total cholesterol measurement may not represent a patient's usual cholesterol concentration. Cholesterol results that are at the decision points should be followed with a repeat measurement. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories and local populations.

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

The EasyRA Chemistry Analyzer flags any result above 600 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 CHOL test to 1200 mg/dL.

An elevated cholesterol result obtained in a POL should be confirmed by a follow-up test in a clinical laboratory.

PERFORMANCE CHARACTERISTICS[5]

Reportable range
The reportable range is 10 to 600 mg/dL. Extended range is 10 to1200 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 cholesterol (y) on the EasyRA Analyzer to the performance of a similar cholesterol reagent (x) on the Roche COBAS MIRA* Analyzer. The data shown below represents single determinations on the EasyRA Analyzer vs. the average of two replicate values obtained on the Roche COBAS MIRA Analyzer.

Number of Samples	53	Range of Samples	11 to 578 mg/dL
Slope	0.97	y Intercept	5.8
Correlation Coefficient	0.9992	Regression Equation	Y = 0.97*X + 5.8

**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 CHOL 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)
Within run imprecision: Five replicates of each of three levels of commercial human serum-based QC material were tested per day over 5 days.

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
215.9	4.1	1.9
168.1	7.8	1.0
105.7	1.3	1.3

Total Imprecision: Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days.

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
168.5	2.5	1.5
302.9	3.6	1.2
106.2	1.3	1.2

Linearity (CLSI, EP6-A)

Linear from 10 to 600 mg/dL, based on the linear regression Y = 1.004*X + 0.025.

Interfering Substances (CLSI, EP-7A)
According to SFBC recommendations, studies have been performed to determine the level of interference from different compounds:
Less than 10% interference was classified as “no significant interference.”
No significant interference was found up to 500 mg/dL hemoglobin
No significant interference was found up to 5 mg/dL bilirubin.
No significant interference was found up to 2250 mg/dL triglycerides (using Intralipid*).
No significant interference was found up to 9 mg/dL ascorbic acid.
No significant interference was found up to 600 mg/dL glucose.

**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.[6,7]

REFERENCES

1. Naito, H.K., Coronary Artery Disease and Disorders of Lipid Metabolism. *Clinical Chemistry:Theory, Analysis and Correlation*, 4th ed. Kaplan, L.A. Pesce, A.J., Kazmierczak, S.C. (Mosby, Inc. eds. St. Louis USA) 2003: 603.
2. Allain, C.C., et al., Enzymatic determination of total serum cholesterol. *Clin. Chem*.,1974:20, 470.
3. Henry, ed. Clinical Chemistry, Principles and Technics, New York, NY, Harper and Row, 1974.
4. 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). NIH publication No. 01-3670: May 2001.
5. Data on file at Medica.

6. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
7. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EasyRA Assay Parameters (CHOL)

Primary Wavelength (nm)	520
Secondary Wavelength (nm)	600
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Yes (with each calibration)
Sample Blank	No
Reaction Time	5.6 min
Calibration interval (maximum)	30 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	3.0
Diluent volume (µl)	20
Reagent volume (µl)	180
Decimal Places (default)	0
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	10 to 600 mg/dL