MEDICA

IVD CE

REF 10216-4 4 x 37 mL

TRIGLYCERIDES (TRIG)

Wedges each contain a usable volume of 37 mL of reagent.

INTENDED USE

The EasyRA TRIG reagent is intended for the quantitative measurement of triglycerides in human serum and plasma (with lithium heparin as anticoagulant) using the "MEDICA EasyRA® Clinical Chemistry Analyzer." Measurements of triglycerides are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, and other diseases involving lipid metabolism or various endocrine disorders.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION[1, 2]

Triglycerides make up 95% of the tissue-storaged fat within the body. Triglycerides are synthesized both in the intestines from dietary fats and in the liver from dietary carbohydrates. An elevated concentration of triglycerides in serum is associated with arteriosclerosis and may be an indicator of various lipid metabolism disorders such as hyperlipoproteinemia, lipase activity deficiency and also diabetes, renal or endocrine disorders.

PRINCIPLE OF THE PROCEDURE

The assay method of measuring triglycerides is via several sequential enzymatic reactions as described by Fossati et. al.[3] and involving a Trinder-type reaction mechanism.[4-6]

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Kinase, Mg++}]{\text{Glycerol}} \text{Glycerol-1-P} + \text{ADP}$$

$$\text{Glycerol-1-P} + O_2 + 2H_2O \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{p-chlorophenol} \xrightarrow[\text{Peroxidase}]{\text{Horseradish}} \text{quinoneimine dye (Red)}$$

The intensity of the red color at the maximum absorbance at 520 nm is proportional to the triglycerides concentration in the sample.

REAGENT

Buffer containing	
Mg++	0.5 mmol/L
p-Chlorophenol	3.0 mmol/L
ATP	2.6 mmol/L
4-Aminoantipyrine	0.4 mmol/L
Lipoprotein lipase(Pseudomon. sp)	>1000 U/L
Glycerol kinase (Cellulomon. sp)	> 400 U/L
G-3-P oxidase (Pediococcus sp)	> 2400 U/L
Horseradish Peroxidase	> 540 U/L

Stabilizers and Preservatives

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 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 COLLECTIONS AND STORAGE[6]
Clear unhemolyzed serum or plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum or plasma as soon as possible after collection. Use serum or plasma from patients who have been fasting for at least 12 hours. At room temperature, phospholipids may hydrolyze releasing free glycerol resulting in an increase in triglycerides in the sample. Serum triglycerides are stable for 10 days at 2 – 8°C and for 3 months at -20°C.

PROCEDURE

Materials Provided
Medica TRIG Reagent Wedge, REF 10216

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 (30 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 EasyRA 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 daily with the assay when 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 triglyceride concentration from the ratio of change in the unknown sample absorbance to the change in absorbance of the calibrator multiplied by the calibrator value.

$$\text{TRIG (mg/dL)} = \frac{\Delta A_{U_{520}} - \Delta A_{U_{700}}}{\Delta A_{C_{520}} - \Delta A_{C_{700}}} \times \text{CalValue}$$

Where ΔA_U and ΔA_C are the change in absorbance values of unknown sample and the calibrator, respectively; and “Cal Value” is the concentration of triglyceride in the calibrator (mg/dL).

Expected Values[7]
The reference range for triglycerides in serum is as follows according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines.

Triglycerides		Primary Target of Therapy
< 150	mg/dL	Normal
150-199	mg/dL	Borderline High
200-499	mg/dL	High
> 500	mg/dL	Very High

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

The EasyRA Analyzer flags any result above 750 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 TRIG test to 1500 mg/dL.

PERFORMANCE CHARACTERISTICS[8]

Reportable Range
The reportable range is 3 to 750 mg/dL Extended range is 3 to 1500 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 TRIG (y) on the EasyRA Analyzer to the performance of a similar TRIG 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 COBAS MIRA Analyzer.

Number of samples	60	Range of samples:	2 to 1150 mg/dL
Slope	0.9945	y Intercept	8.0119
Correlation Coefficient	0.9975	Regression Equation:	Y = 0.9945*X + 8.0119

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

Number of samples	63	Range of samples:	8 to 706 mg/dL
Slope	0.9735	y Intercept	-1.9025
Correlation Coefficient	0.9992	Regression Equation:	Y = 0.9735*X – 1.9025

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

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

Within run imprecision:

QC Level mg/dL	Within Run SD mg/dL	Within Run CV %
252	1.3	0.5
90	0.6	0.6
78	0.7	0.9

Total Imprecision:

QC Level mg/dL	Total Imprecision SD mg/dL	Total Imprecision CV %
252	2.8	1.1
90	1.1	1.2
78	1.6	2.0

Linearity (CLSI, EP6-A)

Linear from 3 to 750 mg/dL, based on the linear regression Y = 0.9794*X + 2.0853.

Limit of Blank (LOB):	1.653 mg/dL	(CLSI, EP17-A)
Limit of Detection (LOD):	2.16 mg/dL	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)
Less than 10% interference was classified as “no significant interference.”
There is significant negative interference at hemoglobin concentrations above 500 mg/dL.
There is significant negative interference to bilirubin above 5.5 mg/dL.
There is significant positive interference from indocyanine green.
Samples containing elevated levels of Immunoglobulin M (IgM) or samples from patients with Waldenstrom’s Macroglobulinemia may produce unreliable results.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[9,10]

REFERENCES

1. Naito, H.K., *Coronary Artery Diseases 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); p 603.
2. *Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP),* JAMA. (2001): 285: p 2486.
3. Fossati P, Prencipe L, *Serum Triglycerides Determined Colorimetrically with an Enzyme that Produces Hydrogen Peroxide*, Clin Chem. (1982) 28: p 2077.
4. Trinder, R. *Ann. Clin. Biochem.* (1969), 6: p 24.
5. Barham, D., Trinder, R., *Analyst.* (1972) 97: p 142.
6. Tietz NW. Editor, *Clinical Guide to Laboratory Tests*, 3rd ed. WB Saunders and Co., Philadelphia, PA, (1995), p. 610-611.
7. 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.
8. Data on file at Medica.
9. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
10. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EasyRA Assay Parameters (TRIG)

Primary Wavelength (nm)	520
Secondary Wavelength (nm)	700
Reaction Type	Special End Point (3)
Reaction Direction	Increase
Reagent Blank	No
Sample Blank	No
Reaction Time	9.6 min
Calibration interval (maximum)	30 days
Reagent on-board stability	30 days

Serum/Plasma

Sample volume (µl)	4.0
Diluent volume (µl)	20
Reagent volume (µl)	190
Decimal Places (default)	0
Units (default values)	mg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	3 to 750 mg/dL