TRIGLYCERIDES MR CE

CONTENTS			
REF	1155005	Triglycerides MR	2 x 50 mL
	1155010	Triglycerides MR	4 x 100 mL
For *in vitro* diagnostic use only			

TRIGLYCERIDES MR

Enzymatic colorimetric method

ENDPOINT

PRINCIPLE

The method[1,2] is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosin triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.
A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2), proportional to the concentration of triglyceride in the sample.

$$\text{Triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\ \text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P} + \text{ADP}$$

$$\text{Glycerol-3-P} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$\text{4-AA} + 4\ \text{Phenol} \xrightarrow[\text{POD}]{H_2O_2} \text{Quinoneimine} + H_2O$$

REAGENT COMPOSITION

R1 **Monoreagent**. PIPES buffer 50 mmol/L pH 6.8, LPL≥ 12 KU/L, GK ≥ 1 KU/L, GPO ≥ 10 KU/L, ATP 2.0 mmol/L, Mg^{2+} 40 mmol/L, POD ≥ 2.5 KU/L, 4-AA 0.5 mmol/L, phenol 3 mmol/L, non-ionic tensioactives 2 g/L (w/v). Biocides.

CAL **Triglycerides standard**. Glycerol 2.26 mmol/L, equivalent to 200 mg/dL of glycerol trioleate. Secondary standard.

STORAGE AND STABILITY

Store at 2-8ºC.
The Monoreagent and Standard are stable until the expiry date stated on the label.
Discard the reagent if presents an absorbance above 0.150 at 500 nm against distilled water or if it fails to recover the declared values of control sera.

REAGENT PREPARATION

The Monoreagent and the Standard are ready-to-use.

SAMPLES

Serum or EDTA plasma obtained by the patient after an overnight fast. Remove from cells within 2 hours of venipuncture. Analyze samples immediately or refrigerate. Stable for 1 week at 4-8ºC.

INTERFERENCES

- Bilirubin levels up to 14 mg/dL interfere.
- Hemolysis (hemoglobin > 0.5 g/dL) does not interfere.
- Glycerol in rubber stoppers or in contaminated glassware will give higher triglycerides values.[3]

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 500 ± 20 nm.
- Constant temperature incubator set at 37ºC.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	Sample	Standard
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
Standars	–	–	10 µL

3. Mix and let stand the tubes 15 minutes at room temperature (16-25ºC) or 5 minutes at 37ºC.
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 1 hour protected from light.

CALCULATIONS

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{mg/dL triglycerides}$$

Samples with concentrations higher than 600 mg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es

If results are to be expressed as SI units apply:
mg/dL x 0.0113 = mmol/L

REFERENCE VALUES [4]

Updated clinical values of triglycerides used to classify risk groups.

Triglycerides	Risk Classification
< 150 mg/dL (< 1.70 mmol/L)	Normal
150-199 mg/dL (1.70-2.25 mmol/L)	Borderline/high
200-499 mg/dL (2.26-5.63 mmol/L)	High
≥ 500 mg/dL (≥ 5.65 mmol/L)	Very high

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of triglycerides. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of triglycerides. Assayed.

CLINICAL SIGNIFICANCE

The plasma level of lipids (triglycerides and cholesterol) and lipid derivates, especially lipoproteins (HDL and LDL), aids in the diagnosis of many metabolic disorders. An imbalance in the level of lipoproteins in plasma leads to *hyperlipoproteinemia*, a group of disorders that affects lipid and lipoproteins levels in plasma, causing coronary heart disease (CHD) and atherosclerosis. Each type of hyperlipoproteinemia is associated with an abnormal elevation of triglycerides, cholesterol or lipoprotein subfraction.

Prospective studies[4] indicate that elevated triglycerides are also an independent risk for coronary heart disease. The finding that elevated triglycerides are an independent CHD risk factor suggest that some triglyceride-rich lipoproteins are atherogenic. The latter are partially degraded VLDL, commonly called *remnant lipoproteins*. In clinical practice, VLDL colesterol is the most readily available measure of atherogenic remnant lipoproteins, and as such can be a target of colesterol-lowering therapy.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 800 mg/dL
- ***Precision***

mg/dL	Within-run			Between-run		
Mean	220	368	512	220	372	490
SD	1.8	2.6	2.4	1.9	2.8	3.7
CV%	0.81	0.7	0.47	0.87	0.27	0.76
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: UVIKON 930 for 8 days.

- ***Sensitivity.*** Using a 1:100 sample/reagent at 505 nm, 1mg c triglyceride will produce a net absorbance of approximately 0.003.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 30 r = 0.996 y = 1.116 + 0.439

REFERENCES

1. Buccolo G and David, H. Clin. Chem. 19 : 476 (1973).
2. Fossati, R. and Prencipe, L. Clin. Chem. 28: 2077 (1982).
3. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).
4. SPECIAL REPORT. 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. 285 : 2486 (2001).

B1155-1/0312
R1.ing

Joaquim Costa, 18, 2ª planta. 08390 Montgat – Barcelona (Spain). Tel. (+34) 93 4694990 Fax (+34) 93 4693435
Almacén: Sant Antoni Mª Claret, 8 bis. 08390 Montgat – Barcelona (Spain) www.linear.es e-mail: info@linear.es