Triglycerides-LQ

GPO-POD. Liquid

Quantitative determination of triglycerides

CE IVD

PACKAGING

Ref.: 101-0444	Cont.: 4 x 250 mL
Ref.: 101-0527	Cont.: 6 x 100 mL
Ref.: 101-0579	Cont.: 3 x 100 mL
Ref.: 101-0594	Cont.: 12 x 50 mL

Store at 2-8ºC

CLINICAL SIGNIFICANCE

Triglycerides are fats that provide energy for the cell.

Like cholesterol, they are delivered to the body's cells by lipoproteins in the blood. A diet with a lot of saturated fats or carbohydrates will raise the triglyceride levels. The increases in serum triglycerides are relatively non-specific. For example liver dysfunction resulting from hepatitis, extra hepatic biliary obstruction or cirrhosis, diabetes mellitus is associated with the increase[3,6,7].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2).

In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample[1,2,3].

REAGENTS

R	GOOD pH 6.3	50 mmol/L
	p-Chlorophenol	2 mmol/L
	Lipoprotein lipase (LPL)	150000 U/L
	Glycerol kinase (GK)	500 U/L
	Glycerol-3-oxidasa (GPO)	3500 U/L
	Peroxidase (POD)	440 U/L
	4 – Aminophenazone (4-AP)	0.1 mmol/L
	ATP	0.1 mmol/L
TRIGLYCERIDES CAL	Aqueous primary standard	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Reagent and calibrator provided are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm $\geq$ 0.26

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1].

Stability of the sample: 5 days at 2-8º C .

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

TRIGLYCERIDES CAL: Proceed carefully with this product because due its nature it can get contamined easily.

LCF (Lipid Clearing Factor) is integrated in the reagent.

Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 505 nm (490-550)
   Cuvette: . . . . . 1 cm light path
   Temperature . . . . . 37º C / 15-25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard[(Note 1,2)] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25º C.
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times \text{Standard conc.} = \text{mg/dL triglycerides in the sample}$$

Conversion factor: mg/dL x 0.0113= mmol/L.

QUALITY CONTROL

Control Sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagent and calibration for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

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REFERENCE VALUES

Men	40 – 160 mg/dL
Women	35 – 135 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit 0.000 mg/dL to linearity limit 1000 mg/dL.
If the concentration is greater than linearity limit dilute 1/2 the sample with ClNa (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	110	224	111	224
SD	0.64	1.01	3.74	7.91
CV (%)	0.58	0.45	3.38	3.52

Sensitivity: 1 mg/dL = 0.0013 (A).

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagent (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.99810.
Regression equation: y= 0.9178x - 0.5426
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed with bilirubin < 170 µmol/L, hemoglobin < 10 g/L[2].
A list of drugs and other interfering substances with cholesterol determination has been reported [4,5].

BIBLIOGRAPHY

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