Reactivos GPL

Barcelona, España

CE

TRIGLYCERIDES

-TRIGLYCERIDES LS- Liquid. GPO-POD

Store at: +2+8ºC.

Presentation:

Cod. SU033 CONT: *R 2 x 50 mL.+ CAL 1 x 5 mL.*
Cod. SU034 CONT: *R 2 x 125 mL.+ CAL 1 x 5 mL.*
Cod. SU035 CONT: *R 4 x 250 mL.+ CAL 1 x 5 mL.*

Procedure

Quantitative determination of triglycerides.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

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 COMPOSITION

R	GOOD pH 7.5	50 mmol/L
	p-Chlorophenol	2 mmol/L
	Lipoprotein lipase (LPL)	150000 U/L
	Glycerolkinase (GK)	500 U/L
	Glycerol-3-oxidasa (GPO)	2500 U/L
	Peroxidase (POD)	440 U/L
	4 – Aminophenazone (4-AP)	0.1 mmol/L
	ATP	0.1 mmol/L
Triglycerides Cal	Triglycerides aqueous primary Calibrator	200 mg/dL.

REAGENT PREPARATION AND STABILITY

All the reagents are ready to use.

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

Do not use reagents over the expiration date.

Triglycerides Cal: Once open is stable up to 1 month when stored tightly closed at 2-8ºC, protected from light and contamination prevented during their use.

Signs of Reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm. $\geq$ 0.14

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8ºC,. Do not use reagents over the expiration date.

SPECIMEN

Serum or plasma[1]. Stability of the sample: 5 days at 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED

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

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions

- Wavelenght : ..... 505 nm. (490-550).
- Cuvette: ..... 1 cm light path.
- Temperature .....37ºC. 15-25ºC.

2. Adjust the instrument to zero with Blank of reagent.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL.)	1.0	1.0	1.0
Calibrator(note1-2) (µL.)	--	10	--
Sample (µL.)	--	--	10

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

CALCULATIONS

$$\text{Triglycerides (mg/dL.)} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times 200 \text{ (Calibrator conc.)}$$

Conversion Factor. mg/dL. x 0.0113 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Normal and Pathological.

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

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES

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

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

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 triglycerides 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].

REAGENT PERFORMANCE

– *Measuring Range*:
From detection limit of 5,85 mg/dL. to linearity limit of 1000 mg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.

– *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (mg/dL)	118	216	119	215
SD	0.67	0.94	2.17	2.91
CV %	0.60	0.43	1.83	1.36

- Sensitivity: 1 mg/dL. = 0.0011 A
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- No interferencess were observed to bilirubin up to 170 µmol/L, hemoglobin up to 10 g/L[1,2].
- Other substances may interfere. A list of drugs and other substances that could interfere has been reported by Young et. al[3,4].

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

1. Buccolo G et al. Quantitative determination of serum triglycerides by use of enzimes. Clin Chem 1973; 19 (5): 476-482.
2. Fossati P et al. Clin. Chem 1982; 28(10): 2077-2080.
3. Kaplan A et al. Tryglycerides. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 437 and Lipids 1194-1206.
4. Young DS. Effects of drugs on Clinl Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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