TRIGLYCERIDES GPO-PAP LS Mono

IVD

Quantitative determination of Triglycerides in Serum or Plasma. Enzymatic colorimetric method (GPO – PAP)

REF CC1302 R1: 4x60 mL R2: 1x3 mL (standard) **REF CC1300** R1: 4x100 mL R2: 1x3 mL (standard)

METHOD AND PRINCIPLE

Lipase (LP) hydrolyzes the Triglycerides to Fatty Acids and Glycerol which, in the presence of Glycerochinase (GK) and ATP, is phosphorylated to Glycerol-3-phosphate and converted by Glycerol-3-phosphate Oxidase (GPO) into Dihydroxyacetonphosphate and H_2O_2. The formed hydrogen peroxide reacts, in the presence of Peroxidase (POD), with 4-Chlorophenol and 4- Aminoantipyrin, giving a red colored compound whose Absorbance, measured fotometrically at 546 nm, is proportional to the concentration of Triglycerides

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acid}$$

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

$$\text{Glycerol-3-phosphate + } 0_2 \xrightarrow{\text{GPO}} \text{Dihydroxyaceton phosphate + } H_2O_2$$

$$2\ H_2O_2\text{+Aminoantipyrine+4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone + HCl + 4 } H_2O$$

CLINICAL SIGNIFICANCE

The plasma level of lipids (triglycerides and cholesterol) and lipid derivate, especially lipoproteins (HDL and LDL), aids in the diagnosis of many metabolic disorders. An imbalance in the level of lipoproteins in plasma leads to hyperlipoproteinemias, a group of disorders that affects lipid and lipoproteins levels in plasma, causing coronary heart disease (CHD) and atherosclerosis. Each type of hyperlipoproteinemias is associated with an abnormal elevation of triglycerides, cholesterol or lipoprotein sub fraction. Prospective studies indicate that elevated triglycerides are also an independent risk for coronary heart disease. The finding that elevated triglycerides are an independent CHD risk factor suggests that some triglyceride-rich lipoproteins are atherogenic. The latter are partially degraded VLDL, commonly called remnant lipoproteins. In clinical practice, VLDL cholesterol is the most readily available measure of atherogenic remnant lipoproteins, and as such can be a target of cholesterol-lowering therapy.

REAGENT COMPOSITION

Reagent (R1)

Good's buffer, pH 7.2	50 mmol/L
4-Chlorophenol	4 mmol/L
ATP	2 mmol/L
Mg^{2+}	15 mmol/L
Glycerokinase (GK)	≥ 0.4 kU/L
Peroxidase (POD)	≥ 2 kU/L
Lipoprotein lipase (LPL)	≥ 4 kU/L
4-Aminoantipyrine	0.5 mmol/L
Glycerol-3-phosphate-oxidase (GPO)	≥ 1.5 kU/L

Reagent (R2)

Standard (Triglycerides)	value on label

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light.

Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Blank Absorbance (Abs) at 546 nm > 0.200 in 1 cm cuvette.

After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

SPECIMEN

Serum, heparin plasma or EDTA plasma

Avoid hemolysate samples. Separate the serum from the clot quickly.

Stability: 2 days at 20 - 25 °C ; 7 days at 4 - 8 °C ; 1 year at - 20 °C

Discard contaminated specimens. Freeze only once.

PROCEDURE

Wavelength:	546 nm (510 – 570)
Temperature:	37°C
Measurement:	against distilled water

Pipette as follow:

Reagent R1	1000 µL
Sample, Std / Cal / H_2O	10 µL

Mix, incubate for 5 minutes and read Absorbances within 60 minutes.

CALCULATION

$$\text{Triglycerides} = \frac{\text{Abs Sample – Abs Blank Reagent}}{\text{Abs Cal/Std – Abs Blank Reagent}} \times \text{Conc. Cal/Std}$$

Conversion factor: Triglycerides [mg/dL] x 0.01126 = Triglycerides [mmol/L]

CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

As an alternative to the standard included in the package, it is possible to use **MTD Diagnostics Calibrator**:

Chemistry Multicalibrator REF CAL1010 (10 x 3 mL)

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Chemistry Control N - REF CNN1010 10 x 5 mL (Level 1)

Chemistry Control P - REF CNP1020 10 x 5 mL (Level 2)

EXPECTED VALUES

Normal:	< 200 mg/dL (< 2.25 mmol/L)
Borderline high:	200 - 400 mg/dL (2.25 - 4.50 mmol/L)
Elevated	400 – 800 mg/dL (4.50 – 9.00 mmol/L)
Very High	> 800 mg/dL (> 9.00 mmol/L)

Epidemiological studies have observed that a combination of plasma triglycerides > 180 mg/dL (> 2.0 mmol/L) and HDL-cholesterol < 40 mg/dL (1.0 mmol/L) predict a high risk of coronary heart disease. Borderline levels (> 200 mg/dL) should always be regarded in association with other risk factors for coronary heart disease.
Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval.
For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples= 20; Average = 55; S.D. = 0.31; CV = 0.58%
High Level: Samples = 20; Average = 448; S.D. = 3.56; CV = 0.80%

ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y = 1.00 x + 0.899 r = 0.9989

SENSITIVITY: 3 mg/dL

LINEARITY: 3 – 1000 mg/dL

SPECIFICITY / INTERFERENCES

No interferences were observed by bilirubin up to 20 mg/dL and by haemoglobin up to 500 mg/dL. For further information on interfering substances refer to Young DS.

NOTES

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
2.Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

PRECAUTIONS

R1 conteins PHENOL 4 mmol/L – CAS 108-95-2 T R23/24/25 (H331 – H301 – H311) - C R34 (H314).
4-AMINOANTIPYRINE 0.5 mmol/L – CAS 83-07-8 Xn R22 (H302).

H301 – Toxic if swalloved
H302 – Harmful if swalloved
H311 – Toxic in contact with skin.
H314 – Causes severe skin burns and eye damage
H331 – Toxic if inhaled

The products do not contain other dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT).
The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.
However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

BIBLIOGRAPHY

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