TRIGLYCERIDES – GPO-PAP

Diagnostic reagent for the in-vitro quantitative determination of Triglycerides in human serum on both manual and automated systems.

REF:V/TG02.025	50 test	REF:V/TG02.100	200 test
REF:V/TG04.025	100 test	REF:V/TG04.125	500 test
REF:V/TG02.050	100 test		

CLINICAL SIGNIFICANCE

Triglycerides are the main lipids present in the human plasma; the others are the cholesterol, phospholipids and nonesterified fatty acids. They are formed in the intestinal mucosa by the esterification of glycerol and fatty acids. Triglycerides measurements are used in the diagnosis and treatment of patients with diabetes mellitus, liver obstruction, nephrosis and other diseases involving lipid metabolism. The measurement of serum triglycerides is important in the diagnosis of hyperlipoproteinemia and in the prediction, detection and monitoring of atherosclerosis.

METHOD PRINCIPLE

GPO-PAP-enzymatic colorimetric method.

The series of the reactions involved in the assay system is as follows:

1. Triglycerides are hemolyzed by lipoprotein lipase (LPL)

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

2. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK).

$$\text{Glycerol phosphate + O2} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

3.The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H2O2).

$$\text{Glycerol-3-phosphate + O2} \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + H2O2}$$

4. In the presence of peroxidase (POD), hydrogen peroxide affects the oxidative coupling of 4-chlorophenol and 4-aminoantipyrine (4AAP) to form a red color quinoneimine dye which is measured at 546 nm.

$$\text{2H2O2 + 4-AAP + 4-chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine Dye+4H2O}$$

REAGENT COMPOSITION

R 1: Standard	- 200 mg/dl(2.29 mmol/L)
R2: Reagent 2	
- Pipes Buffer pH 7.0	- 50 mmol/L
- 4-chlorophenol	- 6.0 mmol/L
- Magnesium aspartate	- >0.5 mmol/L
- Lipase	- >10 KU/L
- Peroxidase	- >2.0 KU/L
- 4-amino-antipyrine	- 1.0 mmol/L
- Glycerol-3-phosphate oxidase	- >3.5 KU/L
- Glycerol kinase	- >750 U/L
- ATP	- 1.0 mmol/L
- Sodium Azide	- 8.0 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

Wearing personnel protective equipment (overall, gloves, glasses,).

- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with
- plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. Triglycerides reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie. Triglycerides reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C. Once opened, the opened vial is stable for 3 months at 2–8°C.

Deterioration
The **Lab.Vie**. Triglycerides reagent is normally clear. Do not use **Lab.Vie**. Triglycerides reagent if it is turbid or if the absorbance is greater than 0.2 at 546 nm.

SPECIMEN COLLECTION AND PRESERVATION

Patients should be fasting for 10 to 14 hours before blood is drawn. Samples must be drawn in a soap and glycerol free collection device. Recommended anticoagulants are EDTA or heparin at levels of 1mg and 0.2 mg/dl whole blood, respectively. Triglycerides in serum samples remain stable for 7 days at 4°C, for 3 months at -20 °C, and for years at -70 °C.

SYSTEM PARAMETERS

Wavelength	546 nm (500 – 550 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	15– 25°C or 37 °C
Incubation time	10 min. at 15–25°C or 5 min. at 37°C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU High 0.2 AU
Sensitivity	5 mg/dl (0.057 mmol/L)
Linearity	1000 mg/dl (11.45 mmol/L)

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl

Mix and incubate for 5 minutes at 37°C or 10 minutes at 15-25°C. Measure absorbance of specimen "A" and standard "A" against reagent blank within 30 minutes.

CALCULATION

Triglycerides concentration (mg/dl) = $\frac{\text{(A specimen)} \times 200}{\text{(A standard)}}$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	155.1	245.8	156	246.5
SD.	2.03	1.85	2.2	1.9
CV. %	1.31	0.75	1.4	0.87

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**. Triglycerides reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.967.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 5 mg/dl (0.057 mmol/L).

Linearity
The reaction is linear up to Triglycerides concentration of 1000 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemolysis
No significant interference from haemoglobin up to 6.0g/dl.
Icterus
Bilirubin levels higher than 171 µmol/L (10 mg/dl) decrease the apparent triglycerides concentration significantly.
Drugs
Of the drugs tested in-vitro, methyldopa and levodopa cause artificially low triglyceride values at the tested drug Level.
Others
Physiological ascorbic acid concentration does not interfere with the test. Ascorbic Acid levels higher than 2.0 mg/dl decrease the apparent Triglycerides concentration significantly.

EXPECTED VALUES

Serum	mg/dl	[mmol/L]
Females	35-135	0.4 – 1.54
Males	40-160	0.45 – 1.82

DYNAMIC RANGE

5 - 1000 mg/dl (0.057 – 11.45 mmol/L).

REFERENCES

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SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		