TRIGLYCERIDES

(GPO /PAP method)

CHEMCount

INTRODUCTION

Triglycerides are built of glycerol molecule esterified with three fatty acids molecules. Triglycerides are delivered with food or synthesized endogenously in liver. Triglycerides stored in adipose tissue constitute a reserve of energy. Elevated triglycerides serum level is a risk factor of atherosclerosis. Triglycerides measurement is useful for hyperlipidemia diagnosis and treatment or for estimation of atherosclerosis progression.

METHOD PRINCIPLE

Colorimetric, enzymatic method with glycerophosphate oxidase.

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

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

$$\text{Glycerol-3-phosphate} + O2 \xrightarrow{GPO} \text{dihydroxy-acetone-phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{TOOS} \xrightarrow{POD} \text{quinoneimine dye} + 2\,H_2O$$

The colour intensity is proportional to the triglycerides concentration.

KIT CONTENTS

Reagent name	MTGL02050M
R1 - Triglycerides reagent	2 x 50 ml
R2 - standard	2 ml

Refer standard value mentioned on the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagent supplied is ready for use.

CONCENTRATIONS IN THE TEST

Buffer PIPES (pH 7.2)	50 mmol/l
4-aminoantipyrine (4-AAP)	0.4 mmol/l
ATP	1.0 mmol/l
Chromogen	2.0 mmol/l
Glycerol kinase (GK)	≥300 U/L
Glycerol-3-phosphate oxidase (GPO)	≥1000 U/L
Peroxidase (POD)	≥500 U/L
Lipoprotein lipase (LPL)	≥2000 U/L
Activators & Stabilizers.	

WARNINGS AND NOTES

For in vitro diagnostic use only.
If the reagent is deteriorated, the working reagent absorbance shall be > 0.5 (read against distilled water, wavelength λ=505 nm) , also turbidity is observed . Moreover it leads failure to recover control values within the acceptable range. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

Automatic analyser or photometer able to read at 505 nm;
Thermostat at 37ºC; General laboratory equipment;

SPECIMEN

Serum, EDTA or heparinized plasma (recommended: heparin lithium, sodium or ammonium salt) free from hemolysis.
Blood should be collected only if the patient has been fasting for minimum of 12 hours. Before blood collection patient should stay in rest position for about 30 minutes. Venous blood is recommended for triglycerides measurement.

Plasma triglycerides values have been reported to be 2% to 4% lower than serum triglycerides values. Serum should be separated from red blood cells as soon as possible after blood collection.
Serum and plasma can be stored up to 3 days at 2-8ºC or 3 months at -20ºC. Nevertheless it is recommended to perform the assay with freshly collected samples

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Programme Sheets are available on request.

Wavelength	505 nm
Temperature	37°C
Cuvette	1 cm

Pipette into the cuvettes:

REAGENT	Blank (B)	Standard (S)	Test (T)
R1 Triglycerides Reagent	1000 µl	1000 µl	1000 µl
Bring upto the temperature of determination. Then add			
Distilled water	10 µl		
R2 - standard		10 µl	
Sample			10 µl

Mix well, incubate for 10 min. at 37ºC or 20 min. at 25-30ºC. Read the absorbance of test A(T) and standard A(S) against blank (B).

CALCULATION

Triglycerides concentration = A (T) / A(S) x Standard concentration

From calculated triglycerides concentration value subtract 0.11mmol/l (10 mg/dl), which corresponds to average amount of free glycerol in serum.

REFERENCE VALUES

25 - 160 mg/dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.
For Fully Automated analyzers by using multicalibrators or triglycerides standard the calibration curve can plot and the same should be prepared every 8 weeks or with change of reagent lot number.

PERFORMANCE CHARACTERISTICS

Sensitivity / Limit of Quantitation: 2.0 mg/dl (0.023 mmol/l).

Linearity: up to 800 mg/dl (9.06 mmol/l). For higher triglycerides concentrations dilute the sample with 0.9% NaCl in the ratio of 1 to 4 and repeat the assay. Multiply the result by 5.

Specificity / Interferences Haemoglobin up to 3.75 g/dl, bilirubin up to 20 mg/dl and ascorbate up to 62 mg/l do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	End point
Wavelength	505 nm
Zero Setting	Reagent blank
Temperature Setting	37°C
Incubation Temperature	37°C
Incubation Time	10 mins.
Delay time	----
Read time	—
No. of Reading	—
Interval time	—
Sample Volume	0.01 ml (10 µl)
Reagent Volume	1.0 ml (1000 µl)
Standard Concentration	Refer standard vial
Units	mg/dl
Factor	—
Reaction slope	Increasing
Linearity	800 mg/dl

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