Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12591	R1	3 x	40	ml
For 180 tests	R2	3 x	20	ml
Cat. No. 12592	R1	3 x	70	ml
For 315 tests	R2	3 x	35	ml
Cat. No. 12593	R1	2 x	160	ml
For 480 tests	R2	2 x	80	ml

Triglycerides GPO/PAP

Enzymatic colorimetric method

Liquid Reagents

Test Principle

Triglycerides + $3H_2O$ —(*LPL*)→ Glycerol + 3 R-COOH

Glycerol + ATP —(*Glycerolkinase*)→ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate+O2 —(*GPO*)→ Dihydroxyacetone+ phosphate+ H_2O_2

$2H_2O_2$+ 4-aminophenazone + ADPS —(*POD*)→ $2H_2O$ + HCl + dye

dye = 4-(p-benzoquinone-monoimino)-phenazone

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide.
The oxidative condensation of ADPS and 4-aminophenazone in the presence of Peroxidase (POD) and hydrogen peroxide produces a rose colored dye which is measured at 550 nm.
The intensity of the colour formed is directly proportional to the triglycerides concentration in the sample.

Concentration of the test

Reagent R1		
PIPES **[Piperazine-1.4-bis (2-ethane-sulfonic acid)]**	50.0	mmol/L
Magnesium ions	0.98	mmol/L
EDTA	0.20	mmol/L
GPO (Glycerophosphate-Oxidase)	5.0	KU/L
Glycerolkinase	5.0	KU/L
4-Aminophenazone	0.5	mmol/L
ATP (Adenosine-tri-phosphate)	1.8	mmol/L
Detergent, preservative.		
Reagent R2		
Potassium hexacyanoferrate(II)	9.5	µmol/L
ADPS **(N-Ethyl-N- (3-sulfopropyl)-3-methoxyaniline)**	2	mmol/L
POD (Peroxidase)	2.8	KU/L
LPL(Lipoprotein lipase)	≥ 2	KU/L
Stabilizer, preservative.		
Standard : The Concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: *liquid*.
Reagent R2: *liquid*.

All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Mix 2 volumes of bottle R1 with 1 volume of bottle R2.
Stability: 4 weeks at 2 - 8 °C.

Note: Don't use if the initial absorbance of the reagent is greater than 0.350 when measured at 550 nm against water.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma is recommended.
2. Avoid anticoagulants containing fluoride or oxalate.
3. The serum should be collected following a 12 hour fasting, and separated from the clot as soon as possible.
4. Serum or plasma may be stored for 1 week at 2 - 8 °C and for 3 months at - 20 °C.
5. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

Calibrator / Standard

MediCal U Cat. No. 15011
Triglycerides STD. Cat. No.16191

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 546 (540 - 560 nm)
Spectrophotometer	550 nm
Cuvette	1 cm light path
Temperature	37 °C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Distilled water	20 µl	--	--
Calibrator / Standard	--	20 µl	--
Sample	--	--	20 µl
Working Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 - 25°C. Read the absorbance (A). The final color is stable for at least 30 min.			

Calculation

$$\text{Conc.}_{\text{Triglycerides}}\,(\text{mg/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc.}_{\text{Cal./STD.}}\,(\text{mg/dl})$$

$$\text{mmol} \underset{0.0114 \times}{\overset{\times 87.5}{\rightleftharpoons}} \text{mg/dl}$$

Linearity

Up to 1000 mg/dl (11.43 mmol/L) .
If the result exceeds 1000 mg/dl, repeat the test using diluted serum (1+4) with sodium chloride solution (0.9 %) and multiply the result by 5.

Medichem Middle East

Aleppo, Syria, E-mail: info@medichem-me.com

Interferences

1. A number of drugs and substances affect the determination of triglycerides. Young, et al, have published a comprehensive list of these substances.[4]
2. The method is not influenced by bilirubin levels up to 12 mg/dl (< 5 %).
3. Detergents can interfere with the action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents.
4. Ascorbic acid: No significant interference up to 100 mg/dl.
5. Hemolysis interferes with the test.

Precaution

The reagent contains sodium azide (0.1 %) as a preservative. Don't ingest. Avoid skin and eye contact. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.

Reference range

Adults	< 200	mg/dl

References

1. Trinder, C. Klin. Chem. klin. Biochem. 8 (1970) 658.
2. Weibhaar, D. Grossau, E. und All., Med. Welt 26 (1975) 387-390.
3. Kubler, W., Symp. der Deutschen Gesellschaft für Lab. Med. Mainz (1973).
4. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.