ANA BIO ISP TRIGLYCERIDES

(GPO-POD Method)

For Miura Instruments

Intended Use
Triglycerides is a reagent kit used for the determination of triglycerides, based on enzymatic method using lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase.

Principle
Glycerol released from hydrolysis of triglycerides by lipoprotein lipase (LPL) is converted by glycerol kinase (GK) into glycerol - 3 – phosphate which is oxidized by glycerol phosphate oxidase (GPO) to dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide (H_2O_2) oxidizes Phenolic chromogen to a red coloured compound. The intensity of the colored complex is directly proportional to the concentration of Triglycerides in specimen.

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

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

$$\text{Gly-3-Phos. + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$H_2O_2 \text{ + Phenolic chromogen} \xrightarrow{\text{POD}} \text{Red colour compound}$$

Components & Concentration of Reagents

Reagent	Component	Concentration
Enzyme Reagent	Goods Buffer PH 7.2	50 mmol/L
	4-Chlorophenol	≥ 4 mmol/L
	4-AAP	≥ 0.1 mmol/L
	GYK	≥ 40 U/L
	Lipase	≥ 2500 U/L
	GPO	≥ 4000 U/L
	POD	≥ 500 U/L
	Stabilizers, excipients & surface active agents	

Reagent storage and stability
The kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label. **DO NOT FREEZE THE REAGENT.**

Reagent Preparation
Liquid reagent ready for use. After opening the reagent is stable for 30 days if closed, stored at 2° - 8°C, and protect from direct light. Do not mix different batches.

Specimen collection and preservation
Blood should be collected in a clean dry container. Avoid the use of plastic or siliconized container which may prolong clotting time. Serum or plasma should be separated from the cells at the earliest possible. For plasma collection following anticoagulants may be used.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- HEPARIN 200 IU/ml of blood

Avoid use of oxalate and Sodium Fluoride as anticoagulant.
Triglycerides are stable for 4 days in neatly separated serum/plasma at 2° - 8°C.

Automation
This kit, though developed and manufactured to be used as manual assay and with I.S.E. Miura Analyzer, can be used also with other analyzers able to meet the specifications indicated in section "Reaction conditions – Test procedure" Application sheets are available for automatic instruments.
All applications not explicitly approved by KDPL. Cannot be guaranteed in terms of performance, and must there be established by the operator.

Calibration
For Calibration use the "Multicalibrator"

Calibration Stability
For the instrumentation series Miura, the calibration is recommended to be done every 10 days.

Materials required but not supplied in the kit
Calibrators and controls

Assay guidelines for Analyzer I.S.E. Miura

Analyte Name	Triglycerides	
Method Code	TGL	
Type	End-Point	
Unit	mg/dl	
Filter F1	505 nm	
Blank in	Use	
Step	Reaction Volume	U.M.
Volume reagent R1	200	µl
Sample Volume	2	µl
Final Incubation	600	Sec.

Normal range
Guidance value for Children : 60 - 170 mg/dl
Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation
Reaction is linear up to 1000 mg/dl. If the Triglyceride value exceeds 1000 mg/dl, then dilute the specimen suitability with normal saline and repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain correct Triglycerides value.

Quality Control
To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, Wavelength setting, Expiration date of reagents and accuracy of prob aspiration.

Accuracy-Recovery
Triglycerides added to a serum matrix containing known amounts of Triglycerides gave an average recovery of 98%.

Interference
The high dilution of the sample with the reagent reduces to a minimum the interference by lipids. Bilirubin below 30 mg/dl does not interfere in the reaction. Haemoglobin interferes at concentrations above 500 mg/dl.

Precision of the Method

Within-run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	mg/dl	83.74	1.45	1.73	20
High	mg/dl	157.40	0.62	0.39	20
Between run					
Range	U.M	Mean	S.D.	C.V.(%)	No. run
Low	mg/dl	83.63	1.56	1.87	60
High	mg/dl	157.36	1.25	0.80	60

Sensitivity
At 505 nm a concentration of 4.0 mg/dl of Triglycerides can estimate.

References

1. Foosati P., et al Clin. Chem 28, 2077 (1982).
2. Henry, J.B., Clinical diagnosis and management by laboratory methods, 18th ed., W.B Saunders, Philadelphia, 1991, p. 204-211.
3. Tietz, N. W., Clinical guide to laboratory tests, 2nd ed., W.B Saunders, Philadelphia, 1994, p. 1073-1091.
4. Young D.S., Effects of drugs on clinical laboratory tests, 3rd ed., AACC Press. Washington, D.C., 1990, p.3-340 3-346.

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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