ANA BIO CLR TRIGLYCERIDES

(GPO – POD Method)

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.

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

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

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

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

Reagents Provided

1. Triglycerides enzyme reagent - Ready to use.
2. Standard - Triglycerides (200mg/dl).

Reagent storage and stability

The kit should be stored at 2° - 8°C and is stable till the expiry date indicated on the label. The reagent and standard are ready-to-use. DO NOT FREEZE THE REAGENT. The reagent should be stored only in the amber bottle provided, to protect it from direct light. DO NOT SHAKE VIGOROUSLY. Over the period of storage, the reagent may develop a light pink colour. This is expected and does not affect the reagent performance. Discard the reagent if the absorbance of the same exceeds 0.300 against distilled water blank at 510 nm. Contamination of the reagent should be strictly avoided. If the reagent develops turbidity discard the reagent.

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.

Assay guidelines for Analyzers

Reaction Type	End Point with standard
Reaction Slope	Increasing
Incubation Time	10 min. at 37°C / 20 min. at RT (25 ° - 30°C)
Wavelength	510 nm (500 - 530 nm)
Blank	Reagent Blank
Blank Absorbance limit	< 0.300 Abs. against distilled water blank
Sample Volume	10 µl (0.01 ml)
Reagent Volume	1000 µl (1.0 ml)
Standard Concentration	200 mg/dl
Factor Calculation	200 mg/dl ÷ Absorbance of standard
Low Normal	60 mg/dl
High Normal	170 mg/dl
Linearity	Up to 1000 mg/dl

Assay guidelines for Manual Procedure

Bring the enzyme reagent and standard to room temperature before performing the assay.

Reagents	Blank	Standard	Sample
Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	10 µl (0.01 ml)	-
Sample	-	-	10 µl (0.01ml)

1. Mix thoroughly and incubate tubes at 37º C for 10 minutes or 20 minutes at room temperature (25° - 30°C).
2. Read the absorbance against reagent blank at 510 nm (500 - 530 nm).
3. The final colour is stable for 30 minutes if not exposed to direct light.

Calculation

$$\text{TGL. Con. in sample (mg/dl)} = \frac{\text{Sample OD.}}{\text{Std. OD.}} \times \text{Con. of Std.}$$

Normal Range

Guidance value : 60 - 170 mg/dl.

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation

1. If the triglycerides value exceeds 1000 mg/dl dilute the specimen with saline (1:1 ratio) and repeat the assay. Multiply the result obtained by 2 to get the correct triglycerides value.
2. Glycerol contamination in glass ware leads to erroneous result.
3. Care should be taken not to touch reagent and sample with fingers, especially after applying hand lotion which may contain glycerin.

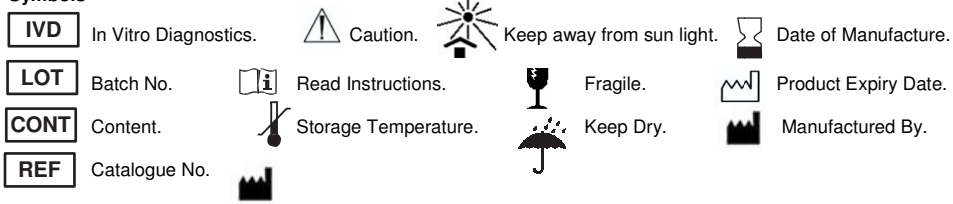
Quality Control

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 and accuracy of pipetting.

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



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