TRIGLYCERIDES(GPO-PAP)

INTENDED USE
Bioline Triglycerides is used for the *In Vitro* quantitative determination of Triglycerides in serum or plasma.

CLINICAL SIGNIFICANCE
Triglycerides are esters of fatty acids and are hydrolyzed to glycerol and free fatty acids. Triglyceride determinations when performed in conjunction with other lipid assays are useful in the diagnosis of primary and secondary hyperlipoproteinemia. They are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction and various metabolic abnormalities due to endocrine disturbances.

METHOD AND PRINCIPLE
Standard methods for the measurement of triglyceride concentrations have involved either enzymatic or alkaline hydrolysis to liberate glycerol. This formulation makes use of the enzymatic hydrolysis and quantification since it is specific and not subject to interference by phospholipids.

The enzymatic reaction sequence employed in the assay of Triglycerides is as follows:

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

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-l-phosphate + ADP

Glycerol- 1-Phosphate + O_2 $\xrightarrow{\text{GPO}}$ DHAP + H_2O_2

H_2O_2 + 4-AA + TOOS $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye + H_2O_2

The present procedure involves hydrolysis of triglycerides by lipase. The glycerol concentration is then determined by enzymatic assay coupled with Trinder reaction that terminates in the formation of a quinoneimine dye. The amount of the dye formed, determined by its absorption at 546 nm, is directly proportional to the concentration of triglycerides in the samples.

REAGENT COMPOSITION
1. Triglyceride Liquid reagent contains the following:
   ATP 0.5 mmol/L, Magnesium acetate 12 mmol/L, TOOS 3.5 mmol/L, 4-Aminophenazone 0.3 mmol/L, Glycerol Phosphate Oxidase > 4500 U/L, Lipase >200,000 U/L, Glycerol kinase >250 U/L, Peroxidase >2,000 U/L, Buffer (pH 7.4) 50 mmol/L, surfactants, stabilizers, and preservatives.
2. Triglyceride standard contains glycerol 200 mg/dl triglycerides as triolein. Sodium azide 0.1% is added as a preservative.

WARNINGS AND PRECAUTIONS
1. For *In Vitro* diagnostic use.
2. Avoid ingestion of reagent as toxicity has not yet been determined.
3. All specimens and controls should be considered infectious and handled appropriately.
4. Reagent and standard contain sodium azide as a preservative. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amount of water to prevent azide build up.

REAGENT PREPARATION
Triglyceride reagent and standard are provided in a ready-to-use form. No preparation is necessary.

REAGENT STORAGE AND STABILITY
Both the Triglyceride reagent and standard must be stored at 2 - 8°C. The reagent may be used until the expiration date indicated on the package label when stored as directed. Protect from direct light. Avoid microbial contamination.

REAGENT DETERIORATION
The reagent should be discarded if:
1. The initial absorbance of the reagent against water is greater than 0.200 when measured at 546 nm.
2. The reagent fails to meet linearity claims or fails to recover stated values. *Note: A yellow or slight purple coloration is normal.*
3. The reagent is turbid or displays evidence of bacterial contamination.

SPECIMEN COLLECTION AND STABILITY
1. Fresh, clear, non-hemolyzed serum from fasting patients is recommended.
2. Triglycerides in serum appear stable for three days when stored at 2 - 8°C.
3. Prolonged storage of the samples at room temperature is not recommended since other glycerol containing compounds may hydrolyze, releasing free glycerol.
4. Blood collection devices lubricated with glycerin (glycerol) should not be used.

INTERFERENCES
Glycerol in rubber stoppers or in contaminated glassware will elevate triglyceride levels. Lipemic or grossly icteric samples will cause falsely elevated results consequently a patient blank should be run. Samples with gross hemolysis or high bilirubin values will produce falsely elevated triglyceride values. A number of drugs and substances affect the measurement of triglyceride.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength : 546 nm
Temperature : 37°C

	Blank	Standard	Sample
Reagent	1.0 mL	1.0 mL	1.0 mL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix and measure the optical density (OD) of standard and sample against the reagent blank after 10 minute of incubation. The final colour is stable for 30 minutes.

CALCULATIONS
Triglycerides results are expressed as mg/dl A = Absorbance

$$\frac{A(\text{sample})}{A(\text{standard})} \times \text{Concn of std} = TG(\text{mg/dl})$$

CALIBRATION
The procedures are calibrated with the standard solution, which is included with each series of tests. Its absorbance is used to calculate the results. It is recommended to establish a linearity curve up to 1000 mg/dl with other available commercial standard solutions to verify the performance of instruments and reagents

LIMITATIONS
The reagent is linear to 1000 mg/dl, specimens above this limit must

be diluted 1:1 with water, reassayed and multiplied the results by two to compensate for the dilution.

QUALITY CONTROL

It is recommended that controls be included in each set of assays.Commercially available control material with established triglyceride values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

Normal <150 mg/dl

It is strongly recommended that each laboratory establish its own normal range

PERFORMANCE CHARACTERISTICS

Linearity: 1000 mg/dl

Sensitivity: Based on an instrument resolution of A = 0.001, this procedure has a sensitivity of 1.3 mg/dl.

Comparison: A group of 91 sera ranging in Triglyceride valuesfrom12 to 1030 mg/dl were assayed by this method and by a similar commercially available reagent. Comparison of the results yielded a correlation coefficient of 0.997 and the regression equation was y =0.946x + 5.373.

Precision:

Within Run

	Serum 1	Serum 2
Mean (mg/dL)	43.2	127.0
Std Dev (mg/dL)	1.19	3.83
CV%	2.78	3.02

Run to Run

	Serum 1	Serum 2
Mean (mg/dL)	42.3	124.1
Std Dev (mg/dL)	1.99	4.12
CV%	4.71	3.32

General Technical Parameters.

Mode	**End Point**
Wavelength (Filter)	**546 nm**
Reaction Direction	**Increasing**
Reagent Blank	**Yes**
Sample Vol.	**10 µL**
Reagent Vol.	**1000 µL**
Incubation Time	**10 min**
Reagent Blank Abs.(Max)	**NMT 0.200 Abs**
Calibration Method	**1- Point**
Standard (Conc.)	**200 mg/dL**
Linearity	**1000 mg/dL**
Decimal Places	**1**
Temp.	**37°C**
Unit	**mg/dL**
Ref Normal	**<150 mg/dl**

REFERENCES

1. Searcy, R.L.: *Diagnostic Biochemistry,* McGraw-Hill, New York (1969).
2. Fossati, P., Principle, L.: *Clin. Chem.* 28:2077 (1982).