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# TRIGLYCERIDE - GPO LIQUID REAGENT

## INTENDED USE
For the *In Vitro* quantitative determination of Triglycerides in serum or plasma.

## INTRODUCTION
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.

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.[1]

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

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

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

$$\text{Glycerol- 1-Phosphate + } 0_2 \xrightarrow{\text{GPO}} \text{DAP + } H_20_2$$

$$H_20_2 + \text{4-AA + DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye + } 2H_20$$

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 520 nm, is directly proportional to the concentration of triglycerides in the samples.[2, 3]

## REAGENT COMPOSITION
1. Triglyceride Liquid reagent contains the following:
   ATP 0.5 mmol/L, Magnesium acetate 12 mmol/L, 4-Chlorophenol 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 with surfactant to yield 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.

## 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 0.500 when measured at 520 nm.
2. The reagent fails to meet linearity claims or fails to recover stated values. *Note: A yellow or pink coloration is normal.*
3. The reagent is turbid or displays evidence of bacterial contamination.

## SPECIMEN COLLECTION
1. Fresh, clear, non-hemolyzed serum from fasting patients is recommended.
2. Triglycerides in serum appear stable for three (3) days when stored at 2 - 8°C.[4]
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.[5]

## MATERIALS PROVIDED
1. Triglyceride GPO Liquid Reagent
2. Triglyceride Standard (200 mg/dl)

## MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer capable of measuring absorbances at 500-550 nm
2. Test tubes and rack
3. Accurate pipetting devices
4. Constant temperature incubator set at 37° C
5. Timer

**GENERAL INSTRUCTIONS**
The reagent for triglycerides is intended for use either as an automated procedure on chemistry instruments or as a manual procedure on a suitable spectrophotometer.

**AUTOMATED PROCEDURE**
Refer to appropriate application manual available.

**MANUAL PROCEDURE**
1. Label tubes: “blank”, “standard”, “control”, “patient”, etc.
2. Pipette l.0 ml of reagent into all tubes.
3. Place all tubes in a 37° C heating block for at least 4 minutes.
4. Add 0.010 ml (10 µl) of sample to respective tubes and mix.
5. Incubate all tubes for five (5) minutes at 37° C.
6. Zero spectrophotometer at 520 nm with reagent blank (Wavelength range: 500-550).
7. Read and record absorbances of all tubes.

Note: Final color is stable for sixty (60) minutes at room temperature.

- TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

**PROCEDURAL LIMITATIONS**
The reagent is linear to 1000 mg/dl (11.4 mmol/L), 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.

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

$$\frac{A\ (patient)}{A\ (standard)} \times \text{Concentration of standard (mg/dl)} = \text{Concentration of patient (mg/dl)}$$

Example:

$$\frac{0.24}{0.31} \times 200 = 154.8\ \text{mg/dl}$$

NOTE: To obtain the results in SI units (mmol/L) multiply the result in mg/dl by 0.0113.

**EXPECTED VALUES**
36 – 165 mg/dl[6]
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 values from 12 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. (Comparison studies were performed according to NCCLS Tentative Guideline, EP9-T.)

**Precision:**

| | **Within-Run** | |
|---|---|---|
| | **Serum 1** | **Serum 2** |
| Mean (mg/dl) | 43.0 | 127.0 |
| Std. Deviation (mg/dl) | 1.19 | 3.83 |
| C.V. (%) | 2.78 | 3.02 |

| | **Run-to-Run** | |
|---|---|---|
| | **Serum 1** | **Serum 2** |
| Mean (mg/dl) | 42.3 | 124.1 |
| Std. Deviation (mg/dl) | 1.99 | 4.12 |
| C.V. (%) | 4.71 | 3.32 |

**REFERENCES**

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