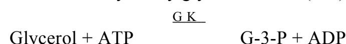
LiquiColor® Triglycerides Test Procedure No. BD386

For the Quantitative Enzymatic- Colorimetric Determination of Triglycerides in Serum or Plasma

Summary and Principle

Measurement of triglyceride levels, when performed in conjunction with other lipid assays, proves useful in the diagnosis of primary and secondary hyperlipoproteinemia. Triglyceride concentrations are also of interest in following the course of diabetes mellitus, nephrosis, biliary obstruction and various metabolic abnormalities resulting from endocrine disturbances.[1, 2, 3]

1. Glycerol and fatty acids are first formed by lipase action on the triglycerides.
2. Glycerol is then phosphorylated by adenosine-5'-triphosphate (ATP) to produce glycerol-3-phosphate (G-3-P) and adenosine-5'-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK):



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

3. The G-3-P is oxidized by glycerylphosphate oxidase (GPO) producing dihydroxyacetone phosphate (DAP) and hydrogen peroxide

$$\text{G-3-P} + O_2 \xrightarrow{GPO} \text{DAP} + H_2O_2$$

4. Peroxide reacts with a 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase (POD) to form quinoneimine

$$2H_2O_2 + \text{4-aminopyrine} + \text{4-chlorophenol} \xrightarrow{POD} \text{quinoneimine} + HCl + 4H_2O$$

Lipid Clearing Factor **(LCF):** a mixture of specially designed additives (please inquire) is integrated into the triglyceride reagent to help minimize interference due to lipemia.

Reagents

Enzymatic Triglyceride Reagent, Cat. No. BD386a/d

When activated: ATP 2.0 mM, Magnesium Salt 5.0 mM, 4-Aminoantipyrine 0.7 mM, m-Hydroxybenzoic Acid 5.0 mM, Glycerylphosphate Oxidase >7000 U/L, Sodium Azide .01% Lipase >200,000 U/L, Glycerol Kinase >1000 U/L, Peroxidase >2000 U/L, Buffer 50 mM, pH 7.3 ± 0.1.

Triglyceride Activator, Cat. No. BD386b

Enzyme concentrate, activators and stabilizers.

Triglyceride Standard, 200 mg/dL, Cat. No. FT7610

Contains glycerol with surfactant to yield 200 mg/dL triglycerides as triolein. Sodium azide 0.1% added as a preservative.

Precautions: For In Vitro Diagnostic Use.
Reagents and standard contain sodium azide as a preservative. May react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Reagent Preparation:

Cat. No. BD3861: Add 9 drops of Triglyceride Activator, Cat. No. BD386b to one bottle of Triglyceride Reagent Cat. No. BD386a (30 mL) or add 50 µL of Activator to every 5.0 mL of Reagent. Invert gently 3-4 times. Before use allow to stand for at least 15 minutes at room temperature.

Cat. No. BD3862: Add 7 drops of Triglyceride Activator to every 30 mL of Triglyceride Reagent Cat. No. BD386d (250 mL) or 50 µL of Activator to every 5.0 mL of Reagent. Invert 3-4 times. Before use allow to stand at least 15 minutes at room temperature.

Mix Triglyceride Activator by gently inverting 2-3 times before use.

Reagent Storage and Stability: Triglycerides reagent is stable until expiration date on label when stored at 2-8°C. Once activated the reagent is stable for 6 weeks at 2-8°C or 3 days at 15-30°C, protected from light. Activator is stable until expiration date on label when stored at 2-8°C. Bring reagents and standard to room temperature before use.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 500 nm (492-530 nm)
Accurate pipetting devices
Heat block or water bath, 37°C
Interval Timer, cuvets

Specimen Collection and Preparation[2]

Sample Stability: Triglycerides are reportedly stable for at least 10 days at 2-8°C. Do not store samples at 15-25°C as phospholipids may hydrolyze, releasing free glycerol and falsely elevating triglyceride values.

Interfering Substances: Blood collecting devices containing glycerol (glycerin) cannot be used, such as those having stoppers so lubricated. Gross hemolysis or high billrubin values, will produce falsely elevated values. A number of drugs and substances affect the determination of triglycerides.

Interference from gross icteric and heavily hemolyzed specimens is correctible by use of serum/plasma blank (refer to "Results" section)

Automated Analyzer

Parameters:

Wavelength .... 500 nm
Reaction Type .... Endpoint
Reaction Direction .... Increasing
Reaction Temperature .... 37°C
Sample/Reagent Ratio 1:100
Equilibration Time .... 3 Seconds
Read Time .... 4 Seconds
Lag Time .... 300 Seconds
Blank Absorbance Limit 0.500A
High Absorbance 2.000A
Standard .... 200 mg/dL
Low Normal .... 30 mg/dL
High Normal .... 150 mg/dL
Linearity .... 1000 mg/dL

Above parameters should be employed in programming automated analyzers for Triglycerides. Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Interchim Customer Service Department.

Manual Procedure

1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Activated Reagent	1.0	1.0	1.0
Standard	–	0.01	–
Sample	–	–	0.01

NOTE: Volumes may be increased 2-fold if the instrument requires volumes greater than 1.0mL.

2. Incubate all cuvets at 37ºC for 5 minutes, or incubate at room temperature for 10 minutes

3. Read S and U vs. RB at 500 nm within 60 minutes.

Quality Control: Ser-T-Fy® I, Normal Control Serum, Cat. No. FT7670 and Ser-T-Fy® II, Abnormal Control Serum, Cat. No. FT7680 are recommended for each run. Other commercially available controls with triglyceride values assayed by this method are also suitable. Triglycerides determined in these materials, by this procedure should fall within the ranges stated for the controls. Two levels of controls should be analyzed with each run.

Results

Values are derived by the following equations:

$$\text{1. Serum Triglyceride (mg/dL)} = \frac{Au}{As} \times 200$$

where Au and As are the absorbance values of unknown and standard, respectively and 200 the concentration of the standard (mg/dL). When a serum blank is required (icteric or hemolyzed specimen), label another tube SB (Step 1. "Procedure" section). Add 1.0 mL "normal" saline, 0.01 mL serum, mix by inversion, transfer to cuvet and read absorbance (Asb) vs distilled water at 500 nm. Use this value to correct that of the unknown as follows:

$$\text{2. Serum Triglycerides (mg/dL)} = \frac{Au - Asb}{As} \times 200$$

NOTE: Samples having triglyceride values greater than 1000 mg/dL are diluted 5-fold (1 + 4) with normal saline (sodium chloride, 8.5 g/L), the assay repeated and results multiplied by the dilution factor of 5.

Expected Values[4]

30 – 150 mg/dL
It is recommended that each laboratory establish its own normal range of expected values, since differences exist between instruments, laboratories, and local populations.

Performance Characteristics[5]

Reproducibility: A study was performed on a control serum (mean = 57 mg/dL) and a patient pool (mean = 327 mg/dL) which entailed 10 determinations on each for 5 successive days. Coefficients of variation (CV) were within run 1.18% and 0.88% and between runs 1.96% and 0.88%, respectively.

Correlation: Determination of triglyceride levels by the procedure described (y) and by the GPO method of Boehringer Mannheim (x) on 57 sera (range 47 to 950 mg/dL) showed a correlation coefficient (r) of .999 and a regression equation of y = 1.029x – 7.46.

Linearity: When performed as directed the method is linear from 0 to 1000 mg/dL.

References

1.Fredrickson DS, Levy RI, lees RS: New Engl J Med 276:34, 1967
2.Dryer RL: IN Fundamentals of Clinical Chemistry. NW Teitz, Ed. WB Saunders, Philadelphia, 1970, p329.
3.Wahlefeld, AW: IN Methods of Enzymatic Analysis, Vol 5, HU Bergmeyer, Ed. Academic Press, New York, 1974, pp 1831-1835.
4.Scheletter G. Nussel E: Arbeitsmed Sozialmed Pracentimed 10:25, 1975
5.BAN Laboratory data

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