POINTE SCIENTIFIC, INC.
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Triglycerides - GPO Reagent Set

Intended Use
For the quantitative determination of triglycerides in serum or plasma.

Method History
Triglycerides have been commonly determined by methods that liberated and then measured Glycerol. Liberation has been performed by either enzymatic hydrolysis or with the use of an alkali.[1,2] The first fully enzymatic method was described by Bucolo and David[3] in 1973. This method was modified to a colorimetric test by Megraw et al[4] in 1979.
The present method uses a modified Trinder[5,6] color reaction to produce a fast, linear, endpoint reaction.[7,8]

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

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} G_3P + \text{ADP}$$

$$G_3P + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{TBHB} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 2H_2O$$

Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G_3P) and adenosine-5-diphosphate in a reaction catalyzed by glycerol kinase (GK). Glycerol-3-phosphate is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3-hydroxy-2,4,6-tribomobenzoic acid (TBHB) in a reaction catalyzed by peroxidase to yield a red colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration of Triglycerides in the sample when measured at 540nm.

Reagents
Triglycerides Reagent (Concentrations refer to reconstituted reagent): ATP 1.0mM, Magnesium Salt > 5.0mM, TBHB 2.0mM, GPO > 2,000 U/L, Lipase > 200,000 U/L, GK 6,000 U/L, Peroxidase > 500 U/L, Buffer, Surfactant, Stabilizers, Fillers, with Sodium Azide 0.1%.

Reagent Preparation
Reconstitute the reagent vial with the volume of water stated on the label.

Reagent Storage
1. Unreconstituted reagent should be stored refrigerated (2-8°C) and is stable until the expiration date on the label.
2. Reconstituted reagent is stable for two days at room temperature (22-28°C) and for 30 days refrigerated (2-8°C). The reagent must be stored in sealed amber, glass containers. Protect from light.

Reagent Deterioration
The reagent should not be used if:
1. Moisture has entered the vial and the powder has a dark discoloration.
2. The reconstituted reagent has an initial absorbance greater than 0.500 against water at 540nm. (A slight pink color is normal).
3. The reagent fails to recover stated values in control sera.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Reagent contains Sodium Azide as a preservative. This 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.

Specimen Collection and Storage
1. Specimens from fasting individuals are recommended.
2. Serum, EDTA, or heparinized plasma may be used as samples.
3. Do not use plasma treated with citrate, oxalate or fluoride.
4. Do not use grossly hemolyzed or highly icteric specimens.
5. Triglycerides in serum are stable for several days when stored at 2-8°C.
6. Do not store samples at room temperature as phospholipids may hydrolyze, releasing free glycerol and falsely elevating Triglyceride values.

Interferences
1. Glycerol in rubber stoppers or as a contaminant in glassware, etc., will elevate values.
2. Samples with gross hemolysis or very high bilirubin values, will produce falsely elevated Triglyceride values.
3. Some detergents interfere with the reaction by producing a precipitate and/or red color. Rinse glassware well.
4. A number of drugs and substances affect the determination of Triglycerides, See Young, et al.[9]

Materials Provided
Triglycerides Reagent.

Materials Required but not Provided
1. Test tubes/rack
2. Accurate pipetting devices
3. Timer
4. Heating block or water bath (37°C)
5. Spectrophotometer able to read at 500-550 nm
6. Distilled water

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Reconstitute reagent vial with distilled water according to instructions.
2. Label tubes: “Blank”, “Standard”, “Patient”, “Control”, etc.
3. Pipette 1.0 ml of reagent in each cuvette.
4. Place all tubes in incubator and bring reagent up to 37°C.
5. Pipette 0.01 ml (10ul) of sample into respective tubes.
6. Incubate all tubes for five minutes at 37°C.
7. Zero spectrophotometer at 540nm with Reagent Blank.
8. Read and record absorbances of all tubes.
9. To obtain values in mg/dl, see “Calculations”.

NOTES:
1. Final color is stable for thirty minutes.
2. Samples with values above 1000 mg/dl should be diluted 1:1 with water, re-run, and results multiplied by 2.

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Limitations
Glycerol (free glycerol and glycerol released upon hydrolysis of triglycerides) is measured by this procedure. Free glycerol levels in serum are generally low, but elevations may be caused by improper storage or sample contamination.

Calibration
Use an appropriate aqueous Triglyceride Standard (200 mg/dl) or serum calibrator.

Calculations
Abs = Absorbance

$$\frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times \text{Concentration of Standard} = \text{mg/dl as triolein}$$

Sample Calculation: If Abs. Sample = 0.300, Abs. of Standard = 0.200, concentration of Standard = 200 mg/dl

$$\frac{0.300}{0.200} \times 200 \text{ mg/dl} = 300 \text{ mg/dl Triglycerides}$$

Note: To obtain values in S.I. Units, multiply mg/dl x 0.0113 = mmo/L.

Quality Control
To monitor the integrity of the reaction; normal and abnormal control serum with known values should be run with patient samples.
Serum based controls with known concentrations of triglycerides should be used to monitor the validity of the reaction. Aqueous or albumin-based controls should not be used since they by-pass the critical "Lipase" stage of the reaction.

Expected Values
36-165 mg/dl
The expected range is taken from reference literature.[10] It is recommended that each laboratory establish its own reference range.

Performance
1. Linearity: 1000 mg/dl
2. Comparison: Studies performed using the present method and a similar GPO-Trinder Reagent yielded a coefficient correlation of 0.999 with a regression equation of y=1.12x – 10.2.
   Sample values ranged from 45 to 609. (N=65).
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
98	.05	.05	99	1.0	1.0
290	2.5	.09	290	2.9	1.0

References

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Rev. 5/03 P803-T7531-01