

Triglycerides Reagent

(Liquid)

Catalog #: 43723

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

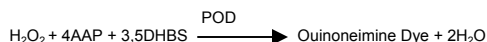
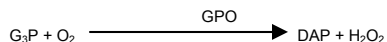
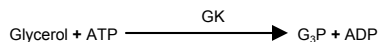
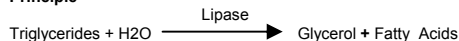
For the *in vitro* quantitative determination of Triglycerides in serum.

METHODOLOGY

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 David³ in 1973. This method was modified to a colorimetric test by Megraw et al⁴ in 1979.

The present method uses a modified Trinder^{5,6} color reaction to produce a fast, linear, endpoint reaction.^{7,8}

Principle



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 (G₃P) 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 3,5 dichloro-2-hydroxybenzene (3,5DHBS) 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.

REAGENT COMPOSITION

Active Ingredients	Concentrations
ATP	2.5 mM
Magnesium Salt	2.6 mM
4-Aminoantipyrine	0.8 mM
3,5DHBS	1.0 mM
GPO (Microbial)	11,000 U/L
Lipoprotein Lipase (microbial)	4,400 U/L
GK (Microbial)	660 U/L
Peroxidase Horseradish	2,900 U/L
Buffer	50 mM
Sodium azide (0.05%) as a preservative	
PH 7.0 ± 0.2	

Precautions

1. 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.
2. This reagent is for *in vitro* diagnostic use only.

REAGENT PREPARATION

Reagent comes in a ready to use form.

REAGENT STORAGE

Store the reagent at 2-8°C (refrigerated).

The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

DO NOT USE THE REAGENT IF:

1. Reagent is turbid
2. The reagent has an absorbance greater than 0.200 against water at 520 nm.
3. The reagent fails to recover stated values in control sera.

SPECIMEN COLLECTION AND STORAGE

1. Serum specimens from fasting individuals are recommended.
2. Do not use grossly hemolyzed or highly icteric specimens.
3. Triglycerides in serum are stable for several days when stored at 2-8°C.
4. Do not store samples at room temperature as phospholipids may hydrolyze, releasing free glycerol and falsely elevating Triglyceride values.

INTERFERENCES

Studies to determine the level of interference for hemoglobin, and bilirubin, were carried out, the following results were obtained:

Hemoglobin:

No significant interference (± 10%) from hemoglobin up to 600 mg/dL.

Bilirubin:

No significant interference (± 10%) from bilirubin up to 4.1 mg/dL.

1. A number of drugs and substances may affect the accuracy of this test. See Young, et al⁹
2. Glycerol in rubber stoppers or as a contaminant in glassware, etc., will elevate values.
3. Samples with gross hemolysis or very high bilirubin values, will produce falsely elevated Triglyceride values.
4. Some detergents interfere with the reaction by producing a precipitate and/or red color. Rinse glassware well.
5. A number of drugs and substance affect the determination of Triglycerides, See Young, et al.⁹

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. An SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters

Triglycerides (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	520 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 100
e.g. Sample Vol.	0.01 mL (10mL)
Reagent Vol.	1.0 mL
INCUBATION TIME:	5 Min

Procedure Notes

1. Final color is stable for fifteen minutes.
2. Samples with values above 800 mg/dL should be diluted 1:1 with water, rerun, and results multiplied by 2.

Calculation

Abs. = Absorbance

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

Abs. Standard of Standard

Sample calculation:

Abs. Sample = 0.300

Abs. of Standard = 0.200

concentration of Standard = 300 mg/dl

$\frac{0.300}{0.200} \times 300 \text{ mg/dL} = 450 \text{ mg/dL Triglycerides}$

NOTE: To obtain values in S.I. units multiply mg/dL x 0.113 = mmol/L

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

Aqueous standards can be used to calibrate the procedure or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Triglycerides values.

EXPECTED VALUES

35 – 160 mg/dL

The expected range is taken from reference literature.¹⁰ Each laboratory should establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 800 mg/dL.

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	47
Range of samples:	8 – 948 (mg/dL)
Correlation Coefficient:	0.9994
Slope:	1.021
Intercept:	-3.5(mg/dL)

Precision:

Within Run	Level 1	Level 2
Mean (mg/dL)	127	141
S.D. (mg/dL)	2.3	1.1
C.V. (%)	1.8	0.8

Total	Level 1	Level 2
Mean (mg/dL)	127	141
S.D. (mg/dL)	3.3	2.8
C.V. (%)	2.6	2.0

Sensitivity:

A calibration factor of approximately 831.55 was obtained, which is equivalent to a sensitivity of 0.0012 D Abs per mg/dL.

REFERENCES

1. Wieland, O., Methods of Enzymatic Analysis, H.O. Bergmayer, Ed., Academic Press pp. 211-214 (1963).
2. Eggstein, M., Kreutz, F.H., Klin. Wochenschr. 44:262 (1966).
3. Bucolo, G., David, H., Clin. Chem. 19:656 (1973).
4. Megraw, R., et al Clin. Chem. 25:273 (1979).
5. Trinder, P., Ann. Clin. Biochem 6:24 (1969).
6. Barham, D., Trinder, P., Analyst 97:142 (1972).
7. Fossati, P., Prencipe, L., Clin. Chem. 28:2077 (1982).
8. McGowan, MW., et al Clin. Chem. 29:538 (1983).
9. Young, D.S., et al, Clin. Chem. 21:1D (1975).
10. Sisson, J.A., Handbook of Clinical Pathology, J.B. Lippincott Co., (1976).

Manufactured for:



23679 Calabasas Road, Unit 241
Calabasas, CA 91302
800-952-2470

PI: 43723.01

Rev. 09/05/06