# LiquiMAX TRIGLYCERIDES - SLR

**(TBHBA/10 Minutes End Point)**
**GPO - PAP TRINDER'S METHOD**

## ORDERING INFORMATION

| Ref. No. | Pack Size | Presentation |
|---|---|---|
| AVTGL - 25 | 25 ml | Single Liquid Reagent |
| AVTGL - 50 | 2 x 25 ml | |
| AVTGL - 100 | 4 x 25 ml | |
| AVTGL - 250 | 5 x 50ml | |
| AVTGL - 500 | 10 x 50ml | |

## INTENDED USE:

LiquiMAX TRIGLYCERIDES is an in-vitro diagnostic kit is use for the quantitative determination of triglycerides in human serum and plasma.

## PRODUCT FEATURES :

1. Single Liquid Reagent.
2. Linearity 1200 mg/dl.
3. With *TBHBA: as Chromogen (*2,4,6-Tribromo-3-hydroxy-benzoic acid).
4. 10 Minutes End Point Assay.
5. Excellent correlation with INT & UV methodologies.

**CLINICAL SIGNIFICANCE:** Triglycerides are esters of the trihydric alcohol glycerol with3 long chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases. The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis. This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and TBHBA under the catalytic action of peroxidase to form a red complex (Trinder endpoint reaction).

## PRINCIPLE:

Triglycerides $\xrightarrow{LPL}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{GK}$ Glycerol - 3 - Phosphate (G - 3 - P)

G - 3 - P + $O_2$ $\xrightarrow{GPO}$ $H_2O_2$ + Dihydroxyacetone phosphate

$H_2O_2$ + 4 - Aminoantipyrine + TBHBA $\xrightarrow{POD}$ Red Quinoneimine Complex + $H_2O$ + Hcl

(*2,4,6-Tribromo-3-hydroxy-benzoic acid).

The triglycerides present in the serum are catabolised into Glycerol and free fatty acids byLipoprotein Lipase. Liberated Glycerol is converted to Glycerol-3-phosphate in presence of Glycerol Kinase and ATP. Glycerol 3 phosphate is acted upon by Glycerol-3-phosphate Oxidase to form Hydrogen Peroxide. This together with Phenolic compound TBHBA and 4-Aminoantipyrine in presence of Peroxidase gives the blue purple colour complex. The intensity of the colour is measured at 505 nm (490-550 nm) and is proportional to the Triglycerides concentration in serum samples. Since TBHBA is a aggressive chromogen the end point is achieved within 5 minutes.

## STORAGE AND STABILITY:

All the reagents must be stored at 2-8$^0$C and are stable till the expiry date mentioned on the labels. When opened contamination must be avoided.

## KIT COMPONENTS

1. Triglycerides Reagent.
2. Triglycerides Standard : Concentration as stated on the label.

## COMPOSITION

| | | |
|---|---|---|
| 1. | LPL | - > 5 KU/L |
| 2. | Glycerol Kinase | - > 1.25 KU/L |
| 3. | Glycerol Phosphate Oxidase | - > 5 KU/L |
| 4. | Peroxidase | - > 2 K U/L |
| 5. | ATP | - > 2 m mol/L |
| 6. | 4AAP | - > 10 m mol/L |
| 7. | TBHBA | - > 0. 2 m mol/L |
| 8. | GOODS BUFFER | - > 20 m mol/L |
| 9. | Surfactants and stabilizers | |

## REAGENT RECONSTITUTION & STABILITY

The Triglycerides is ready to use and stable up to expiry mention on the label & contamination is avoided.

## MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

## REAGENT DETERIORATION

The reagents included in the kit are ready to use. A slight red colour (up to 0.20 Abs Units) against distilled water blank at 505 nm does not affect the performance of the reagents.

## WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP.

## SPECIMEN COLLECTION AND STORAGE

Serum/EDTA Plasma.
Do not use hemolysed samples.

## SYSTEM PARAMETERS:

| | | |
|---|---|---|
| Reaction Mode | : | End Point |
| Units | : | mg/dl |
| Wave Length | : | 505 nm (490-550) |
| Blanking with | : | Reagent |
| Flow Cell Temp. | : | 37$^0$C |
| Sample Volume | : | 10 µl |
| Reagent Volume | : | 1000 µl |
| Linearity | : | 1200 |
| Standard Conc. | : | 200 |
| Low Normal | : | 0 |
| High Normal | : | 150 |

## TEST PROCEDURE:

Pipette into test tubes labelled Blank (B), Standard (S) and Test (T) as follows:

| Reagent | B | S | T |
|---|---|---|---|
| Triglycerides Reagent | 1.0 ml | 1.0 ml | 1.0 ml |
| Triglycerides Standard (Conc:200 mg/dl) | – | 10 µl | – |
| Specimen | – | -- | 10 µl |

Mix well and incubate for 10 minutes at 37$^0$ C or for 15 minutes at R.T.

Mix and read absorbance of Standard (S) and Test (T) against Blank (B) at 505 nm or with green filter (490-550nm).

## CALCULATIONS

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs. Of T}}{\text{Abs. Of S}} \times 200$$

## EXPECTED VALUES

Serum Triglycerides : Up to 150 mg/dl

It is recommended that laboratories should establish their own normal range.

## QUALITY CONTROL & CALIBRATION:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality Control material checks both, the instrument and the reagent function.

## PERFORMANCE CHARACTERISTICS

### 1. Linearity

Linearity : 1200 mg/dl.

### 2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 2 mg/dl.

### 3. Interferences

No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 mg/dl.

### 4. Precision:

Reproducibility was determined using human samples the following results were obtained:

**Intra-Assay**

| N=20 | Mean (mg/dl) | SD (mg/dl) | CV% |
|---|---|---|---|
| Control serum 1 | 122 | 1.09 | 0.89 |
| Control serum 2 | 150 | 1.79 | 1.19 |
| Control serum 3 | 206 | 1.44 | 0.70 |

**Inter-Assay**

| N=20 | Mean (mg/dl) | SD (mg/dl) | CV% |
|---|---|---|---|
| Control serum 1 | 121 | 1.96 | 1.62 |
| Control serum 2 | 161 | 1.84 | 1.14 |
| Control serum 3 | 204 | 2.36 | 1.16 |

### 5. Method Comparison:

A comparison of the LiquiMAX Triglycerides - SLR (y) with a commercially obtainable assay (x) gave with 40 samples the following result:

$y = 0.897 x – 1.149$; $r = 0.9685$

## LIMITATIONS

Measuring range: 2-1200 mg/dl. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

## WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

## NOTES:

1. Do not use recycled plastic tubes as they react with TBHBA Chromogen leading to the false results. Always use soap and glycerol free glass tubes.
2. Contamination of Standard and Reagents must be avoided. After use all the reagents must be immediately stored back at 2-8$^0$C.
3. Replug the Triglycerides Standard vial after use. Use clean glassware & microtips while pipetting Triglycerides standard.
4. Contamination by soap or glycerol will affect this assay.
5. No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 Mg/dl triglycerides.
6. For sample values higher than 1200 mg/dl, dilute the sample with normal saline and multiply the result with appropriate dilution factor.
7. As with all the diagnostic procedures, the physician should evaluate the data obtained by he use of this kit in light of other clinical information.

## REFERENCES:


1. Jacobe, N.J., Van Demark, P.J. (1960) Arch Biochem. Biophys. 88, 250.
2. Trinder, P. (1960) Amn. Clin. Biochem., 6,24.
3. Bucolo G., David M. Clin. Chem 19,476 (1973).


## Symbols Used on Pack

| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Warning/Caution |
| LOT | Batch No. | IVD | In vitro diagnostic device |
| | Manufacturing Date | | Storage Limit |
| | Expiry Date | | Consult instruction for use |
| | Manufacturer | | Keep away from sunlight |
| | Keep Dry | | Do not use if package is damaged |
| | Contains sufficient no. of test | | |

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