TRIGLYCERIDES

(GPO-POD Method) **Liquid Reagent**

INTENDED USE:

This reagent kit is used for in-vitro quantitative determination of Triglycerides in human serum and plasma.

TEST PRINCIPLE:

Triglycerides are enzymatically hydrolysed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate. Glycerol-3phosphate is oxidised to dihydroxyacetone phosphate (DAP) by glycerolphosphate oxidase producing hydrogen peroxide (H_2O_2) . In a Trinder(5) type colour reaction catalyzed by peroxidase, the H₂O₂ reacts with 4-aminoantipyrine (4-AAP) and ESPAS to produce a purple coloured dye. The absorbance of this dye is proportional to the concentration of triglycerides present in the sample.

REACTION:

Triglycerides +
$$H_2O$$
 \xrightarrow{Lipase} Glycerol + Free Fatty acids

Glycerol + ATP $\xrightarrow{Glycerol\ Kinase}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 \xrightarrow{GPO} DAP + $2H_2O_2$
 H_2O_2 + 4-AAP + ESPAS \xrightarrow{POD} Quinoneimine dye + $2H_2O_2$

KIT CONTENTS:

Reagent 1: Triglycerides Enzyme Reagent

Reagent 2: Triglycerides Standard (200 mg/dl)

Product Insert : 01 No.

PREPARATION OF THE WORKING REAGENT:

All the reagents are ready to use.

STORAGE AND STABILITY:

All the reagents should be stored in 2-8°C and are stable till the expiry date mentioned in the labels.

SPECIMEN COLLECTION AND STORAGE:

Serum (fasting) is preferred to plasma. Heparinised plasma may be used. Samples should be used on the same day. If necessary they may be preserved at 2-8°C for upto 4 days.

PRECAUTIONS /

- 1. Storage conditions as mentioned on the kit to be adhered.
- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- 3. Before the assy bring all the reagents to room temperature.
- 4. After use store the kit contents immediately as 2-8°C.
- 5. Avoid contamination of the reagent during assay process.
- 6. Use clean glassware free from dust or debris.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual):

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

Pipette into Test Tube	Blank	Standard	Test
Triglycerides Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 μΙ	-
Sample	-	-	10 μΙ

Mix well and incubate for 10 minutes at 37°C.

Read absorbance of Standard (A_s), and Test (A_T) against Blank (A_R) at 546 nm or with green filter (505-546nm).

CALCULATIONS:

Triglycerides Conc. in mg/dl =

Abs of
$$A_T$$
- A_B
Abs of A_S - A_B
X 200 (Conc. of Standard)

NORMAL VALUES*:

Serum-Plasma: 60-170 mg/dl

*It is recommended that each laboratory should establish its own normal range representing its patient population.

PERFORMANCE:

1. Linearity: 800 mg/dl

Comparison:

A comparison between Triglycerides Liquid Reagent (y) and a commercially available test (x) using 65 samples gave the following results:

$$y = 1.112x - 6.702$$

r = 0.99

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
Low	85.0	1.4	1.4	85.0	1.8	2.0
High	195.0	1.4	0.4	285.0	6.4	0.9

4. Specificity: No interference upto Hemoglobin 1gm/l & Bilirubin 30 mg/dl.

CLINICAL SIGNIFICANCE:

Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates. Measurement of triglycerides is important in the diagnosis and management of hyperlipidaemias. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus, and endocrine disturbances.

Elevation of triglycerides has been identified as a risk factor for atherosclerotic disease.

AUTOMATED APPLICATIONS:

Triglycerides Liquid reagents can be used with Hitachi 700 series, RA50, 1000 XT, Express 550, Syncron CX4, LISA 200, BTR 810/820/830. Erbachem-5, Ranlab etc.

Application sheets for use on specific semiautomatic / batch analysers are available on request.

Input parameters for semiauto / auto analysers are given below:

INPUT PARAMETERS	VALUES		
Type of reaction	End point		
Wavelength	546 nm.		
Incubation time	10 minutes		
Standard concentration	200		
Units	mg/dl		
Temperature	37°C		
Upper Normal value	170 mg/dl		
Lower Normal value	60 mg/dl		
Linearity	800 mg/dl		
Working reagent volume	1.0 ml		
Sample/Standard volume	10 μΙ		

QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

REFERENCES:

- 1. Product Data Sheet, Triglyceride G Code No 99769801, Wako Pure chemical Industries Ltd., Dallas TX.
- 2. McGowan MW, et. al. Clin Chem 1983;29: 538.
- 3. Fossati P, Prencipe L. Clin Chem 1982:28: 2077-80.
- 4. Trinder P. Ann Clin Biochem 1969:6:24-7.
- 5. Klotzsh, S.G and Me Namara, R.J Clin Chem 1990:36:1605-13.
- 6. Young DS, Effects of Drugs on Clinical Laboratory Test. Third Edition. 1990:3:19-25.

