TRIGLYCERIDES (TRIGS)

Liquid Reagent
GPO-PAP Method
MANUAL

INTENDED USE
For the quantitative *in vitro* determination of Triglycerides in serum or plasma. This product is suitable for Manual use.

Cat. No.

TR 1697	R1a. Buffer	4 x 100 ml
4 x 100 tests	R1b. Enzyme Reagent	4 x 1.7 ml
	CAL. Standard	1 x 5.5 ml

GTIN: 05055273206586

CLINICAL SIGNIFICANCE [1]
Triglyceride measurements are used in the diagnosis and treatment of diseases involving lipid metabolisim (Hyperlipoproteinaemia), various endocrine disorders (diabetes mellitus neprosis) and liver obstruction (extrahepatic biliary obstruction).

COLORIMETRIC METHOD
The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

PRINCIPLE [2, 3, 4]

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

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

SAMPLE PREPARATION [1]
Serum, free of haemolysis.

Triglycerides are stable in serum for 7 days at +2 to +8°C. [6]

REAGENT COMPOSITION

Contents		Concentrations in the Test
R1a.	**Buffer**	
	Pipes Buffer	40 mmol/l, pH 7.4
	4-chlorophenol	5.4 mmol/l
	Magnesium ions	5.0 mmol/l
	ATP	1.0 mmol/l
	Peroxidase [EC 1.11.1.7; Horseradish, 25°C]	≥ 0.5 U/ml
	Glycerol kinase [EC 2.7.1.30; Microbial, 25°C]	≥ 0.4 U/ml
	Glycerol-3-phosphate oxidase [EC 1.1.3.21; Microbial, 25°C]	≥ 1.5 U/ml
	Sodium Azide	0.05 %
R1b.	**Enzyme Reagent**	
	4-Aminoantipyrine	0.4 mmol/l
	Lipases [EC 3.1.1.3; Microbial, 25°C]	≥ 150 U/ml
	Sodium Azide	0.05 %
CAL.	**Standard**	See lot specific insert

SAFETY PRECAUTIONS AND WARNINGS
For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1a contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety data sheets are available on request.

Please dispose of all Biological and Chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS
Buffer and enzyme reagent are stable up to the quoted expiry date when stored unopened at +2 to +8°C.
Transfer the entire contents of one vial of Enzyme Reagent R1b to one bottle of Buffer R1a, rinsing bottle R1b several times, or for a small number of tests pipette **250 Tl** of Enzyme Reagent R1b into **15 ml** of Buffer R1a and mix. The working reagent (R1) is stable for 3 weeks at +2 to +8°C, or for 3 days at +15 to +25°C, protected from light.

NOTE
Prior to use allow the working reagent to stand for at least 15 min. at room temperature.

Standard
Triglyceride standard is ready to use. Stable up to expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED
Buffer
Enzyme Reagent
Triglyceride Standard

MATERIALS REQUIRED BUT NOT PROVIDED
Pipetting devices suitable for the delivery of 10 Tl and 1 ml volumes.
Timing device and heating block or water bath to maintain a temperature of 25°C or 37°C.
NaCl (0.9% W/V) Solution for sample dilution.
Spectrophotometer with wavelength capability of 500 nm.
Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532).

PROCEDURE

Wavelength:	500 nm
Cuvette:	1cm light path
Reaction Temperature:	20-25°C or 37°C
Measurement:	Against Reagent Blank. Only one Reagent Blank per series is required.

Pipette into test tubes:

	Reagent Blank µl	Standard µl	Sample µl
Sample	-	-	10
Standard	-	10	-
Reagent (R1)	1000	1000	1000

Mix, incubate for 10 minutes at 20-25°C or 5 minutes at 37°C. Measure the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank within 60 minutes.

CALCULATION OF THE TRIGLYCERIDE CONCENTRATION

$$\text{Triglyceride concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard conc. (mmol/l)} = \text{mmol/l}$$

$$\frac{A_{sample}}{A_{standard}} \times \text{Standard conc. (mg/dl)} = \text{mg/dl}$$

CALIBRATION

Randox triglyceride standard provided with this kit is recommended for calibration. The standard is prepared gravimetrically using commercially available pure glycerol. Recalibration is recommended for each series of samples run.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

LIMITATIONS

This method does not correct for free glycerol and therefore high levels of free glycerol if present in a sample will elevate test results. To correct for free glycerol, subtract 10 mg/dl (0.11 mol/l) from the triglyceride value obtained. [6]

A list of interfering substances and conditions known to affect triglyceride levels *in vivo*, is given by both Young *et al*.[8] and Friedman *et al*.[9] No representation is made by Randox Laboratories Ltd regarding the completeness of these lists or the accuracy of the information contained therein.

REFERENCE VALUES [7]

The NCEP (American National Cholesterol Education Program) has established the following classification for triglyceride levels according to the risk of developing coronary heart diseases:

Normal < 150 mg/dl
Borderline-high 150 – 199 mg/dl
High 200 – 499 mg/dl
Very High ≥ 500 mg/dl

INTERFERENCE

The method is not influenced by:

Haemoglobin	< 525 mg/dl (5.25 g/l)
Free Bilirubin	< 30 mg/dl
Conjugated Bilirubin	< 23 mg/dl

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [10]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

LINEARITY

The test is linear up to a triglyceride concentration of 11.4 mmol/l or 1000 mg/dl. Dilute samples above this concentration 1+4 with 0.9% NaCl solution and reassay. Multiply the result by 5.

SENSITIVITY

It is recommenced that each laboratory establishes its own range of sensitivity, as this is limited by the sensitivity of the spectrophotometer used. Under the conditions of the assay, a change of 0.1 Absorbance units is equivalent to 0.15 mmol/l.

REFERENCES

1. Jacobs NJ, Van Denmark, P.J., Arch Biochem. Biophys. 1960; 88 250-255.
2. Koditscheck L K, Umbreit, W.W., J Bacteriol 1969; 98: 1063-1068.
3. Bucola G. David H: Clin. chem 1973; 19:476.
4. Wahlefeld A: Methods of enzymatic analysis, 2nd English edition, Hill. Bergmayer, ed. New York, Academic Press Inc, 1974 p 1831.
5. Trinder P, Ann. Clin Biochem 1969; 6: 24-27.
6. Tietz NW: Clinical Guide to Laboratory tests Philaelphia, W B Saunders Co, 1963, pg 484.
7. ATP III Guidelines At-A-Glance Quick Desk Reference, National Cholesterol Education Program, National Institute of Health Publication No. 01-3305, May 2001.
8. Young D.S *et al* Clin Chem, 1975 21:No 5: 1D-432D.
9. Friedman E.B *et al* : Clin Chem 1980 : 26 No 4: 1D-476D.
10. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

The presence of a vertical bar in the margin indicates a Technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

Revised 05 Sep 22 ld