TRIGLYCERIDES - L

Enzymatic colorimetric method for the quantitative determination of Triglycerides in serum or plasma

IVD

ORDER INFORMATION

REF	Kit size
GA4815 00	12x50 ml
KL4815 00	6x60 ml
BK4815 00	6x60 ml

INDICATION

Triglycerides determination is used for the diagnosis and monitoring of lipidic dysfunction for the evaluation risk of the atherosclerotic disease. Recent studies have demonstrated that high levels of triglycerides, accompanied to an increase of low density lipoproteins (LDL), constitute a particular elevated risk for "coronary heart disease" (CHD). High triglycerides concentrations are present in several kidney, liver and pancreas diseases.

METHOD PRINCIPLE

Glycerol, released from triglycerides after hydrolysis with lipoproteinlipase, is transformed by glycerolkinase into glycerol-3-phosphate which is oxidized by glycerolphaphate oxidase into dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, the hydrogen peroxide oxidizes the chromogen ESPT (4-aminophenazone/N-ethyl-methylanilin-propan-sulphonate sodic) to form purple quinoneimine whose colour intensity, measured at 550 nm, is proportional to the concentration of triglycerides in the sample.

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

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

$$\text{Glycerol-3-phosphate + } 0_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$2\ H_2O_2 + \text{Amminoantipirine + ESPT} \xrightarrow{\text{POD}} \text{Quinoneimine + HCl + 4 } H_2O$$

COMPOSITION

REAGENT A:

Good Buffer pH 7.2	50 mmol/l
ESPT	4 mmol/l
ATP	2 mmol/l
Mg^{++}	2 mmol/l
Lipoproteinlipase (LPL)	≥ 1 kU/l
Glycerol kinase (GK)	≥ 0.4 kU/l
Glycerolphosphate oxidase (GPO)	≥ 1.5 kU/l
4-Amminoantipirine	0.5 mmol/l
Peroxidase (POD)	>1 kU/l
NaN_3	≤ 0.095 g/l

STANDARD:	1x5 ml
Glycerol	200 mg/dl
NaN_3	≤ 0.095 g/l

Verified against NIST reference material.

Preparation

Reagents are liquids ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light.

The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, heparin or EDTA plasma.

Stability: 2 days at 20-25 °C
7 days at 2-8 °C
1 year at - 20 °C

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use controls with known triglycerides concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H_2O	10 µl		
Standard	-	10 µl	-
Sample	-	-	10 µl
Mix and incubate for 10 minutes at room temperature (20-25 °C) or for 5 minutes at 37 °C. Read the absorbance (A) of the standard and samples at 550 (540-560) nm against Blank. Colour is stable for 60 minutes, protected from light.			

Note:

- Reaction volumes can be proportionally changed.
- For concentration > of 1000 mg/dl (11 mmol/l) dilute sample 1:5 with NaCl (9 g/l) solution and multiply the result by 5.

CALCULATION OF RESULTS

$$\text{Triglycerides, mg/dl} = \frac{\text{A sample}}{\text{A standard}} \times 200$$

Conversion factor

Triglycerides [mg/ml] x 0.01126 = Triglycerides [mmol/l]

REFERENCE VALUES

(on fasting)

Recommended values:	< 200 mg/dL (2.3 mmol/l)
Upper limit:	200-400 mg/dl (2.3-4.5 mmol/l)
High values:	> 400 mg/dl (4.5 mmol/l)

Epidemiological studies have revealed that a combination of triglycerides > 180 mg/dl (> 2.0 mmol/L) in plasma and cholesterol HDL < 40 mg/dl (< 1.0 mmol/l) cause high risk of CHD. Limit levels (> 200 mg/dl) should be evaluated together with others risk factors for CHD.

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different total cholesterol concentration. The obtained results are reported in the following table:

		Within-run		Between-run	
Sample	**Mean (mg/dl)**	**SD**	**%CV**	**SD**	**%CV**
Serum 1	125.3	3.22	2.6	3.35	2.7
Serum 2	219.1	4.65	2.1	9.19	4.2
Serum 3	715.3	6.60	0.9	39.94	4.9

Linearity

The assay is linear up to 1000 mg/dl (11.3 mmol/l).

Sensitivity

Test sensitivity, in terms of limit of detection, is 1 mg/dl (0.01 mmol/l).

Correlation

A correlation study comparing the present method with a commercial one gave the following results:

y = 1.1922x - 6.5745 mg/dl r = 0.9996

Interferences

Bilirubin	> 40 mg/dl
Hemoglobin	> 250 mg/dl
Ascorbic acid	> 6 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
2. Cole TG, Klotzsch SG, McNamara J: Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p.115-26.
3. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998; 19: 1434-503.
4. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. Biochimica Clinica, 1994; 18, 10:673.
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
6. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC…NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).

AMS S.p.A. – Registered Office and Plant
Via E. Barsanti 17/A
00012 Guidonia (Rome) – Italy
Ph. + 39 0774 354441 r.a.
Fax + 39 0774 578035
www.ams-analyzers.com
info@ams-analyzers.com

Diagnostics Manufacturing
Via Galileo Galilei, 38
Seggiano di Pioltello (MI) - Italy
Ph. + 39 02 929189.1
Fax + 39 02 929189.39
www.gdsrl.com
infodiagnostics@ams-analyzers.com

Met. GA481500.0 eng
Edition: 2011/10/05