ISE S.r.l. Triglycerides – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD

R1: 4x 17,5 mL **R3330000025** REF

Diagnostic reagent for quantitative in vitro determination of triglycerides in human serum or plasma on photometric systems

TEST PARAMETERS

Method:	Colorimetric, enzymatic, GPO – PAP, endpoint, increasing reaction
Wavelength	500 nm, Hg 546 nm
Temperature:	20 - 25 °C or 37 °C
Sample:	Serum, heparinized or EDTA-plasma
Linearity:	up to 1000 mg/dL (11.3 mmol/L)
Sensitivity:	Lower limit of detection: 2 mg/dL (0.02 mmol/L)

SUMMARY [1,2]

Triglycerides are esters of glycerol with three fatty acids and are the most abundant naturally occurring lipids. They are transported in plasma bound to apolipoproteins forming very low density lipoproteins (VLDL) and chylomicrons. Measurement of triglycerides is used in screening of the lipid status to detect atherosclerotic risks and in monitoring of lipid lowering measures. Studies have shown that elevated triglyceride concentrations combined with increased low density lipoprotein (LDL) concentrations constitute an especially high risk for coronary heart disease (CHD). High triglyceride levels also occur in various diseases of liver, kidneys and pancreas.

TEST PRINCIPLE

Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

Triglycerides $\xrightarrow{LPL}$ Glycerol + fatty acid

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

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

2 H_2O_2 + Aminoantipyrine + 4-Chlorophenol $\xrightarrow{POD}$ Quinoneimine + HCl + 4 H_2O

REAGENT COMPOSITION

COMPONENTS	CONCENTRATIONS	
Good´s Buffer, pH 7.2	50	mmol/L
4 Chlorophenol	4	mmol/L
Mg^{2+}	15	mmol/L
ATP	2	mmol/L
Glycerolkinase (GK)	≥ 0.4	kU/L
Peroxidase (POD)	≥ 2	kU/L
Lipoprotein lipase (LPL)	≥ 2	kU/L
4-Aminoantipyrine	0.5	mmol/L
Glycerol-3-phosphate-oxidase (GPO)	≥ 0.5	kU/L

REAGENT PREPARATION

The reagent is ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	protect from light! Avoid contamination! close immediately after use. do not freeze the reagent!
Storage:	at 2 - 8°C
Stability:	up to the expiration date

Note: The measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

SAMPLE STABILITY AND STORAGE

Stability [4]:	at 20 - 25 °C	2 days
	at 4 - 8 °C	7 days
	at -20 °C	at least one year

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

STANDARD

(not included in the kit – has to be ordered separately)

Concentration:	200 mg/dL (2.25 mmol/L)
Storage:	2 – 8 °C
Stability:	up to the expiration date

Close immediately after use! Avoid contamination!

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard/Calibrator	-	10 µl	-
Dist water	10 µl	-	-
Mix. Incubate 10 minutes at 37 °C or 20 minutes at 20 – 25 °C. Measure absorbance of sample and std./cal. within 60 minutes against the reagent blank.			

CALCULATION

Triglycerides [mg/dL] = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Std./Cal.}}$ x Conc. Std./Cal. [mg/dL]

To correct for free glycerol, subtract 10 mg/dL (0.11 mmol/L) from the triglycerides value calculated above.

UNIT CONVERSION

mg/dL x 0.01126 = mmol/L

REFERENCE RANGE [2] *

	mg/dL	mmol/L
Desirable (fasting):	< 200	2.3
Borderline high:	200 – 400	2.3 – 4.5
Elevated:	> 400	4.5

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Clinical Interpretation [3]

Epidemiological studies have observed that a combination of plasma triglycerides > 180 mg/dL (> 2.0 mmol/L) and HDL-cholesterol < 40 mg/dL (1.0 mmol/L) predict a high risk of CHD. Borderline levels (> 200 mg/dL) should always be regarded in association with other risk factors for CHD.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

The test has been developed to determine triglycerides concentrations within a measuring range from 2 – 1000 mg/dL (0.02 – 11.3 mmol/L). If values exceed this range, samples should

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be diluted 1+4 with NaCl solution (9 g/L) and the result multiplied by 5.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 2 mg/dL (0.02 mmol/L).

PRECISION (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	55.5	0.301	0.54
Sample 2	212	1.69	0.80
Sample 3	447	3.09	0.69

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	88.9	0.795	0.89
Sample 2	235	3.61	1.54

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	3 mg/dL
Bilirubin, conjugated	30 mg/dL
Bilirubin, unconjugated	9 mg/dL
Hemoglobin	500 mg/dL

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between this Triglycerides (y) and a commercially available test (x) using 95 samples gave following results: y = 0.969 x – 0.092 mg/dL; r = 0.9999.

CALIBRATION

The assay requires the use of a Triglycerides Standard or Calibrator.

QUALITY CONTROL

All control sera with Triglycerides values determined by this method can be used.

Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. The reagent contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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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. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p.46-7
5. Young DS. Effects of Drugs on Clinical laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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