TG Color

GPO/PAP AA

Enzymatic method for the determination of triglycerides in serum or plasma

SUMMARY

Triglycerides are lipids absorbed from the diet and also those endogenously produced from carbohydrates. Their measurement is important for the diagnosis and control of hyperlipemia. These diseases may have genetic origin or be secondary to others such as nephrosis, diabetes mellitus and endocrinous dysfunctions. The increase of triglycerides has been identified as a risk factor for atherosclerotic diseases.

PRINCIPLE

The reaction system is as follows:

$$\text{triglycerides} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol + fatty acids}$$

$$\text{glycerol + ATP} \xrightarrow{\text{GK}} \text{glycerol-1-P + ADP}$$

$$\text{glycerol-1-P + } O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{dihydroxyacetonephosphate}$$

$$2\ H_2O_2 + \text{4-AP + chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine}$$

PROVIDED REAGENTS

A. Reagent A: vials containing lipoprotein lipase, glycerol kinase (GK), glycerol phosphate oxidase (GPO), peroxidase (POD), adenosine triphosphate (ATP), and 4-aminophenazone (4-AP).
B. Reagent B: Good buffer solution containing chlorophenol pH 7.5.
S. Standard: 2.26 mmol/l glycerol solution (it is equivalent to 2 g/l triolein).

Final concentrations

Good	50 mmol/l; pH 7.5
Chlorophenol	2 mmol/l
Lipoprotein lipase	≥ 800 U/l
GK	≥ 500 U/l
GPO	≥ 1500 U/l
POD	≥ 900 U/l
ATP	2 mmol/l
4-AP	0.4 mmol/l

NON-PROVIDED REAGENTS

Wiener lab.'s **Calibrador A plus**.

INSTRUCTIONS FOR USE

Standard: ready to use.
Working Reagent:
- 5/10 x 20 ml: add 20 ml Reagent B to a Reagent A vial. Mix until complete dissolution. Homogenize and date.
- 4 x 50 ml: dissolve the content of an Reagent A vial with a part of Reagent B. Transfer to the Reagent B bottle washing several times. Homogenize and date.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable in refrigerator (2-10ºC) until the expiration date shown on the box. Do not expose to high temperatures for long periods of time.
Working Reagent: stable for 30 days in refrigerator (2-10ºC).

INSTABILITY OR DETERIORATION OF REAGENTS

The Working Reagent may show a slight pink coloration that does not affect its performance.
Blank readings over 0.160 O.D. or abnormally low Standard readings indicate Reagent deterioration. Discard in such case.

SAMPLE

Serum or plasma
a) Collection: obtain serum or plasma after 12-14 hours fasting. Separate red blood cells within 2 hours after extraction.
b) Additives: when using plasma, it is recommended to use Wiener lab **Anticoagulante W** or heparin for collection.
c) Known interfering substances: samples with intense hemolysis or markedly icteric samples should not be used as they produce falsely increased values.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: triglycerides in the sample are stable for 3 days in refrigerator (2-10ºC). Do not freeze.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes and pipettes for measuring the stated volumes
- Tubes or spectrophotometer cuvettes.
- Water bath at 37ºC.
- Stopwatch.

ASSAY CONDITIONS

- Wavelength: 505 nm in spectrophotometer or 490-530 nm in photocolorimeter with green filter.
- Reaction temperature: 37ºC
- Reaction time: 5 minutes
- Sample volume: 10 ul

- Reagent volume: 1 ml
- Final Reaction volume: 1.01 ml

PROCEDURE

Samples must be homogenized before use, particularly when dealing with extremely turbid sera.

In three photocolorimeter tubes or spectrophotometric cuvettes labeled B (Blank), S (Standard), and U (Unknown) place:

	B	S	U
Sample	-	-	10 ul
Standard	-	10 ul	-
Working Reagent	1 ml	1 ml	1 ml

Mix. Incubate 5 minutes at 37°C or 20 minutes at room temperature (18-25°C). Let cool and read in spectrophotometer at 505 nm or in photocolorimeter with green filter (490-530 nm) setting the instrument to zero absorbance with distilled water.

STABILITY OF FINAL REACTION

Final reaction color is stable for 60 minutes, thus absorbance should be read within that period.

CALCULATIONS

Correct readings with Reagent Blank and use these readings for calculations.

$$\text{Triglycerides (g/l)} = U \times \text{factor; factor} = \frac{2 \text{ g/dl}}{S}$$

UNITS CONVERSION

Triglycerides (g/l) = 0.01 x Triglycerides (mg/dl)
Triglycerides (mg/dl) x 0.0113 = Triglycerides (mmol/l)

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known triglycerides concentration.

REFERENCE VALUES

The National Cholesterol Education Program (NCEP) expert panel provides the following Triglycerides values:

Recommended: < 1.50 g/l
Slightly high to high: 1.50-1.99 g/l
High: 2.00-4.99 g/l
Very high: ≥ 5.00 g/l

However, each laboratory should establish its own references values.

PROCEDURE LIMITATIONS

See Known interference substances under SAMPLE.
Reducing agents decrease the color response, while oxidants color the Reagent increasing Blanks. Contaminations with glycerol produce falsely increased values.

PERFORMANCE

a) Reproducibility: when replicates of the same sample were simultaneously assayed in 10 different days, the following values were obtained:

Level	S.D.	C.V.
1.14 g/l	± 0.021 g/l	1.82 %
7.41 g/l	± 0.074 g/l	2.11 %

b) Recovery: adding known amounts of triolein to different sera, for the whole linearity range of the method, a recovery between 99.2 and 100.7% was obtained.

c) Linearity: the reaction is linear up to 10 g/l triglycerides. For higher values, dilute 1:2 with saline solution and repeat the test. Multiply the obtained result by dilution performed.

d) Detection Limit: it depends on photometer used. In spectrophotometer, for a 0.001 O.D. absorbance variation, the minimum concentration variation detected for the given assay conditions, will be of approximately 0.008 g/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use. For calibration use Wiener lab's **Calibrador A plus** according to the analyzer's requirements.

WIENER LAB PROVIDES

- 5 x 20 ml (Cat. 1780107).
- 10 x 20 ml (Cat. 1780101).
- 4 x 50 ml (Cat. 1780105).

REFERENCES

- Fossati, P - Clin. Chem. 28/10:2077 (1982).
- McGowan, M.W.; et al - Clin. Chem. 29/3:538 (1983).
- Tietz, N.W. - Fundamentals of Clin. Chem. - W.B., Saunders Co. - Philadelphia, Pa. (1970), pág. 329.
- Expert Panel of National Cholesterol Education Program - JAMA 285/19:2486 (2001).
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

CE — This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

EC REP — Authorized representative in the European Community

IVD — "In vitro" diagnostic medical device

Σ — Conteins sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Cont. — Contents

LOT — Batch code

Manufactured by:

Xn — Harmful

Corrosive / Caustic

Xi — Irritant

Consult instructions for use

Calibr. — Calibrator

CONTROL — Control

CONTROL + — Positive Control

CONTROL − — Negative Control

REF — Catalog number

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