TRIGLYCERIDES (GPO/PAP)

Enzymatic Colorimetric Determination of Serum Triglycerides

REF. TRI-MC – 0530 (5 X 30 ml) TRI-MC – 03100 (3x100 ml)

INTENDED USE

NS Biotec triglycerides reagent is intended for the in vitro quantitative determination of triglycerides in serum and plasma on both automated and manual systems.

CLINICAL SIGNIFICANCE

Triglycerides are esters of the trihydric alcohol glycerol with 3 long chain fatty acids. They are the main lipids present in human plasma; the others are cholesterol, phospholipids, and non-esterified fatty acids. Triglycerides are synthesized in the intestinal mucosa by the esterification of glycerol and free fatty acids. They are then released into the mesenteric lymphatics and distributed to most tissues for storage. Triglycerides are the main storage lipids in humans, where they constitute about 95% of adipose tissue lipids. Elevated levels of triglycerides have been associated with high risk in severe atherosclerosis. High triglycerides levels and hyperlipidemia in general can be an inherited trait or can be secondary to disorders including diabetes mellitus, nephrosis, biliary obstruction, and metabolic disorders associated with endocrine disturbances.

ASSAY PRINCIPLE

Triglycerides are generally determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of glycerol released. The most commonly used methods involve alkaline hydrolysis and either chemical or enzymatic measurement of glycerol. Chemical means of analysis generally rely on measurement of the product of periodate oxidation of glycerol. Eggstein and Kreutz developed an enzymatic method for measuring glycerol released from triglycerides by alkaline hydrolysis. This method was based on the coupled reaction sequence catalyzed by glycerol kinase, pyruvate kinase, and lactate dehydrogenase. A method for complete enzymatic hydrolysis to triglycerides avoiding the need for serum pretreatment was described by Bucolo and David, using a combination of lipase and at least one proteolytic enzyme. Wahlefeld reported that certain esterases could be combined with a lipase to achieve complete triglycerides hydrolysis. Both methods employed a coupled enzymatic reaction sequence to measure glycerol. NS Biotec triglycerides reagent combines the use of lipoproteinlipase, glycerol kinase, and glycerol phosphate oxidase with the peroxidase/4-chlorophenol/4-aminoantipyrine system of Trinder for the measurement of triglycerides in human serum. The series of reactions involved in the assay system are as follows:

1. Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids.
2. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK).
3. The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2).
4. In presence of peroxidase (POD), the hydrogen peroxide (H_2O_2) formed effects the oxidative coupling of 4 - chlorophenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

Triglycerides $\xrightarrow{LPL}$ **Glycerol+ fatty acids**

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

Glycerol-3-phosphate + O_2 $\xrightarrow{GPO}$ **Dihydroxyacetone phosphate+**

2 H_2O_2+ 4-AAP + 4-Chlorophenol $\xrightarrow{POD}$ **Quinoneimine dye + 4 H_2O**

The intensity of the color produced is directly proportional to triglycerides concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Males:	40 – 160 mg/dl (0.45 – 1.82 mmol/l)
Females:	35 – 135 mg/dl (0.4 – 1.54 mmol/l)

For the recognition of the risk factor hyper-triglyceridemia. The following limits are recommended:

Suspicious	>150 mg/dl (1.71 mmol/l)
Elevated	>200 mg/dl (2.28 mmol/l)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the triglycerides results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Triglycerides standard	200 mg/dl
	Pipes buffer, pH 7.8	50 mmol/l
R_2	p-Chlorophenol	2.0 mmol/l
	Lipoprotein lipase	1500 U/l
	Glycerolkinase	800 U/l
	Glycerol phosphate oxidase	4000 U/l
	Peroxidase	440 U/l
	4-Aminoantipyrine	0.4 mmol/l
	ATP	0.3 mmol/l
	Mg2+	40 mmol/l
	Sodium cholate	0.2 mmol/l

Reagent Preparation & Stability

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

Serum or plasma* from fasting patients.

- The only accepted anticoagulants are heparin and EDTA.

Specimen Preparation & Stability

Patients should refrain from eating for 10 to 14 hours before blood is drawn. Samples must be drawn in a soap and glycerol free collection device.

Blood should be collected by venipuncture, after the patient has been in a seated position for at least 5 minutes. Tourniquet usage should be kept to a minimum and the specimen should be allowed to clot for 30 minutes at room temperature[8].

The best specimen is unhemolysed serum, and should be analyzed on the day of collection. Specimens are stable for 7 days when stored at 4^{0}C; several months at –20^{0}C and for years at –70^{0}C[1].

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	10 µl	……
Specimen	……	……	10 µl

Mix, incubate for 5 minutes at 37^{0}C or 10 minutes at 20-25^{0}C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

The color is stable for 60 minutes.

- **Automated Procedure**

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the triglycerides concentration by using the following formulae:

Triglycerides Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \text{ X Standard}$$

- **Unit conversion**

mg/dl x 0.0114 = mmol/l

LINEARITY

When run as recommended, the assay is linear up to 900 mg/dl (20.7 mmol/l).

If result exceeds 900 mg/dl (10.26 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

SENSITIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 3.0 mg/dl (0.034 mmol/l).

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established triglycerides values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**

The only acceptable anticoagulants are heparin and EDTA.

- **Bilirubin:**

No interference from free bilirubin up to level of 10 mg/dl and from conjugated bilirubin up to a level of 12 mg/dl.

- **Drugs:**

Methyldopa and noramidopyrine causes artificially low triglycerides values at the tested drug level. For a more comprehensive review of drugs affecting triglycerides assays refer to the publication by Young

- **Haemoglobin:**

No interference from haemoglobin up to a level of 600 mg/dl.

- **Lipemia:**

Extremely lipemic samples can produce a normal triglycerides result (triglycerides grater than 3000 mg/dl).

- **Others:**

Ascorbic acid levels higher than 2.0 mg/dl decrease the apparent triglycerides concentration significantly.

WARNING & PRECAUTIONS

- NS Biotec triglycerides reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The reagent blank will not exceed an absorbance of 0.06 but don't use the reagent if it is turbid or if the absorbance is greater than 0.2 at 500 nm.
- Extremely lipemic specimens can produce a normal result. Dilute specimens' 1+4 with saline and reassayed. Multiply the result by 5.

BIBLIOGRAPHY

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4. **Eggstein, M & Kreutz,**
5. **F (1966):** Klin, Wschr. 44: 262-267.

	Consult Instruction for Use
	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
	Use By

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