POINTE SCIENTIFIC, INC.
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Triglyceride (GPO) (Liquid) Reagent Set

Intended Use
For the *in vitro* quantitative determination of Triglycerides in serum or plasma.

Clinical Significance
Triglycerides determinations are of interest in the diagnosis and treatment of atherosclerosis, poorly controlled diabetes mellitus, nephrosis, liver disease, or other diseases involving lipid metabolism.

Test Summary
The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati[1] who coupled the reaction with the classical Trinder[2] reaction sequence. This single reagent procedure quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions. This approach is the basis for this method.

Principle
Lipase
Triglycerides ----------➢ Glycerol + Fatty Acids

GK
Glycerol + ATP ----------➢ Glycerol-1-phosphate + ADP

GPO
Glycerol - 1 - phosphate + O_2----------➢DAP + H_2O_2

POD
H_2O_2 + 4-AA +4 -Chlorophenol ----------➢ Quinoneimine Dye + HCL + $2H_2O$

Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate. The glycerol-1-phosphate is then oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The condensation of hydrogen peroxide with 4-chlorophenol and 4-aminophenazone (4-AA) in the presence of peroxidase (POD) produces a red colored quinonimine dye which absorbs at, or near 500nm. The intensity of the colored complex formed is directly proportional to the triglycerides concentration of the sample.

Reagent Composition
4-Chlorophenol 3.5mM, ATP >0.5mM, Magnesium salt 10 mM, 4-Aminophenazone 0.3mM, Glycerol Kinase (microbial) >250 U/L, Glycerol Phosphate Oxidase (microbial) >4500U/L, Peroxidase (horseradish) > 2000 U/L, Lipase (microbial) >200,000 U/L, buffer (pH 7.3 ± 0.1), surfactants, stabilizers, and preservatives, including sodium azide (0.01%).

Reagent Preparation
The reagent is ready to use.

Reagent Storage and Stability
Store the reagent at 2-8°C. The reagent is stable until the expiration date appearing on the label when stored as directed. Protect from direct light. Avoid microbial contamination.

Do not use the reagent if:
1. The initial absorbance of the reagent is greater than 0.350 when measured at 500nm against water in a cuvette with a one centimeter path length.
2. The reagent is turbid or displays evidence of bacterial contamination.

Precautions
1. This reagent set is intended for *in vitro* diagnostic use only.
2. The reagent contains sodium azide (0.01%) as a preservative. Do not ingest. Avoid skin and eye contact. Sodium azide may react with copper or lead plumbing to form explosive metal azides. Upon disposal flush with large amounts of water.
3. All specimens and controls should be handled as potentially infectious. Use safe laboratory procedures. (NCCLS M29-T2)[3]

Specimen Collection and Storage
1. Fresh, clear, unhemolyzed serum is the specimen of choice. The specimen should be collected following the guidelines of NCCLS document H4-A3.[4]
2. The serum should be collected following a 12 hour fast, and separated from the clot as soon as possible. Avoid anticoagulants containing fluoride or oxalate.
3. Serum of plasma may be stored for one week at 2-8°C or for three months at –20°C.[5]
4. Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

Interferences
1. A number of drugs and substances affect the determination of triglycerides.[6,7] Young, et al[8] have published a comprehensive list of these substances.
2. The method is not influenced by hemoglobin values up to 100mg/dl or by bilirubin levels up to 12mg/dl (<5%).
3. Detergents can interfere with the action of lipase. Care should be taken to avoid contamination of laboratory equipment with detergents.

Materials Provided
Triglycerides (GPO) reagent

Materials Required but not Provided
1. Accurate pipetting devices for delivering required sample and reagent volumes.
2. Test Tubes and racks
3. Timer
4. Heating block or water bath (37°C)
5. Spectrophotometer able to read at 500 nm
6. Triglycerides standard or calibrator

Procedure (Automated-General)

Wavelength:	500nm
Assay Type:	Endpoint
Sample/Reagent Ratio:	1:101
Reaction Direction:	Increasing
Temperature:	37°C
Incubation Time:	300 sec.
Low Normal:	44mg/dl
High Normal:	148mg/dl

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Instrument Application Procedures describing the use of this reagent on automated analyzers are available. Please contact our Technical Service department for specific information.

Test Procedure (Manual)

1. Label test tubes: "Blank", "Calibrator/Standard", "Patient", "Control", etc.
2. Pipette 1.0 ml of reagent into the appropriate tubes and pre-warm to 37°C.
3. Add 0.010ml (10ul) of the appropriate sample to their respective tubes. Swirl gently to mix.
4. Incubate all tubes for five (5) minutes.
5. After incubation, zero the spectrophotometer with "Blank" tube, at 500nm. (500-520 nm is acceptable).
6. Read and record the absorbance (Abs.) of all the tubes. The final color is stable for at least 60 minutes.

Limitations

The procedure is linear to 1000 mg/dl (11.3 mmol/L).
Specimens above this limit must be diluted 1:1 with saline and reassayed. Multiply the result by 2 to compensate for the dilution.

Calibration

Use an NIST-traceable Triglycerides standard or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Quality Control

Standard practice for Quality Control should be applied to this procedure. Commercially available controls (2 levels) should be used to monitor the daily acceptable variations. Controls should be assayed at the beginning of each shift, whenever a new lot number of reagent is used, or following any instrument maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable" range established by the laboratory.

Calculation

Triglycerides results are expressed as mg/dl or mmol/L.

$$\text{Triglycerides} = \frac{\text{Abs Unk}}{\text{Abs Std}} \times \text{Conc. Std}$$

Example:
Abs Unk = 0.243
Abs Std = 0.310
Conc. Std = 200 mg/dl

$$\text{Triglycerides} = \frac{0.243}{0.310} \times 200 \text{ mg/dl}$$

Triglycerides = 157 mg/dl

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0113.

Expected Values

44-148 mg/dl (0.50-1.67 mmol/L)[9]

Due to a wide range of conditions (dietary, geographical, age, etc.) believed to affect normal ranges, it is recommended that each laboratory establish its own reference range.

Performance

1. Assay range: 0-1000mg/dl (0-11.3 mmol/L). Samples that exceed 1000 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: A comparison was made between this method and a similar GPO method using 167 samples ranging from 41 mg/dl to 1026 mg/dl. The correlation coefficient was 0.999. Linear regression analysis gave the following equation: This method y = 0.97 x – 4.5 Sy.x = 5.84.
3. Precision: Precision studies were performed following a modification of the guidelines which are contained in NCCLS document EP5-T2.[10]

Within Day			Day to Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
62.6	1.14	1.82	59.1	1.12	1.90
162.4	1.73	1.07	158.1	2.63	1.66
301.9	3.24	1.07	299.3	3.65	1.22

4. Sensitivity: The sensitivity for this product was investigated by reading the change in absorbance at 500nm for a saline sample, and serum samples with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, this product showed little or no drift on a zero sample. Under the reaction conditions described, 1mg/dl of triglycerides gives an absorbance of 0.001.

References

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7. Constantino, N.V., Kabat, H., Am. J. Hosp. Pharm. 30:24 (1973).
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