Pointe Triglyceride (GPO) Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the *in vitro* quantitative determination of Triglycerides in serum or plasma using the Yumizen C560 analyzer. **Rx Only**.

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
$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-1-phosphate + ADP}$$

$$\text{Glycerol - 1 - phosphate + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2$$

$$H_2O_2 + \text{4-AA +4 -Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine Dye + HCL + } 2H_2O$$

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. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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 and Hazards
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]

Hazards:
Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture. **Refer to the Safety Data Sheet for this product (SDS-T7532) available by calling 1-734-487-8300.**

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.

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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. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

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 Pointe Chemistry Calibrator (Catalog Number C7506-50). The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

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. Correlation: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	Triglycerides
N	129
Mean Triglycerides (mg/dL)	199.0
Range (mg/dL)	1-835
Standard Deviation	210.5
Regression Analysis	y = 0.966x – 8.6
Correlation Coefficient	0.9937

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[10]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	89.8	197.1	947.6
Standard Deviation	0.6	4.8	4.7
Coefficient of Variation (%)	0.7%	2.4%	0.5%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	89.1	192.9	953.6
Standard Deviation	2.5	5.3	17.8
Coefficient of Variation (%)	2.8%	2.7%	1.9%

4. Sensitivity**:** 2SD limit of detection (95% Conf) = 0 mg/dL

References

1. Fossati, P., Lorenzo, P., Clin. Chem. 28:2077 (1982).
2. Trinder, P., Ann. Clin. Biol. Chem. 6:24 (1969).
3. NCCLS Document M29-T2, 2nd. Ed. (1991).
4. NCCLS Document H4-A3, 3rd. Ed. (1991).
5. Tietz, N.W., Textbook of Clinical Chemistry, Philadelphia, PA, WB Saunders Co. p888 (1986).
6. Martin, E., Hazards of Medication, Philadelphia, PA, J.B. Lippincott Co. pp.169-189 (1971).
7. Constantino, N.V., Kabat, H., Am. J. Hosp. Pharm. 30:24 (1973).
8. Young, D.S., 3rd Ed. AACC Press, Washington DC (1990).
9. Rifkin, B.M., JAMA 250:1869 (1983).
10. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

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CHEMISTRY PARAMETERS

Chem:	TRIG	No.:	230	Sample Type:	Serum
Chemistry:	Triglycerides			Print Name:	TRIG
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	505			Sec Wave:	660
Unit:	mg/dL			Decimal	0
Blank Time:	10	12		Reaction Time:	49 51

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	1.5 ul	--- Ul	--- ul	R1: 150 ul	--- ul
Decreased:	--- ul	--- Ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- Ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0	1000		Linearity Limit:
Linearity Range (Decreased)	____	____		Substrate Depletion:
Linearity Range (Increased)	____	____		Mixed Blank Abs:
R1 Blank Abs:	____	____		Uncapping Time
Blank Response:	____	____		Reagent Alarm Limit:
Twin Chemistry:				☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	TRIG	0	mg/dL
Chemistry Calibrator	*	*	*	TRIG	*	mg/dL

Calibration Setup

Chem:	TRIG		
Calibration Settings			
Math Model:	Two-Point Linear		
Factor:		Replicates:	2
Acceptance Limits			
Cal Time:	336	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		
Auto Calib.			

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-T7532-480

Manufactured by
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2°C 8°C IVD

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only