

Intended Use

For the *in vitro* quantitative determination of Triglycerides in serum or plasma using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

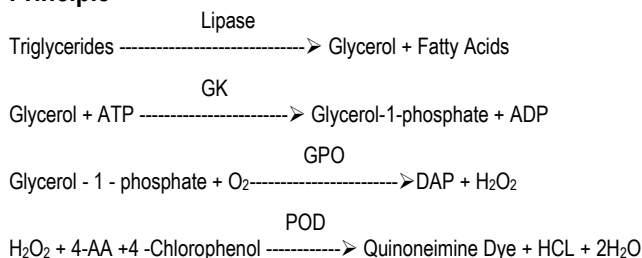
Clinical Significance

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 Fossati¹ who coupled the reaction with the classical Trinder² 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



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:

- 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.
- The reagent is turbid or displays evidence of bacterial contamination.

Precautions

- This reagent set is intended for *in vitro* diagnostic use only.
- 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.
- All specimens and controls should be handled as potentially infectious. Use safe laboratory procedures. (NCCLS M29-T2)³

Specimen Collection and Storage

- Fresh, clear, unhemolyzed serum is the specimen of choice. The specimen should be collected following the guidelines of NCCLS document H4-A3.⁴
- 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.
- Serum of plasma may be stored for one week at 2-8°C or for three months at -20°C.⁵
- Frozen samples should be thawed at room temperature and mixed completely before analysis. Thawed samples should not be refrozen.

Interferences

- A number of drugs and substances affect the determination of triglycerides.^{6,7} Young, et al⁸ have published a comprehensive list of these substances.
- The method is not influenced by hemoglobin values up to 100mg/dl or by bilirubin levels up to 12mg/dl (<5%).
- 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

- Yumizen C230 / Yumizen C240 Analyzer
- Yumizen C230 / Yumizen C240 Operation manual
- Pointe Chemistry Calibrator, catalog number C7506-50
- Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	TRIG	Chemistry:	Triglyceride
Chemistry No.:	230	Print Name:	Triglyceride
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	510 nm	Sec. Wave:	670 nm
Decimal:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	18 20
Unit:	mg/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	2	uL	uL	200	uL uL
Decreased;		uL	uL		
Increased;		uL	uL		

Linearity Range (Standard); 0-1000	Linearity Limit:
Linearity Range (Decreased):	Substrate Depletion:
Linearity Range (Increased):	Mixed Blank Abs.: - 40000 40000
R1 Blank Abs.: - 40000 40000	On-board Stability: 30 Day (s)
Blank Response - 40000 40000	Reagent Alarm Limit: 5
Twin Chemistry:	

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:	
Range:	Flag:

Pointe Triglyceride (GPO) Reagent Set

Slope Offset:	Slope 1	Offset 0	Unit mg/dL
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Pretreatment:	
Pretreat Sample Vol.: uL	Pretreat Reagent Vol.: uL

Ref. Range:			
Sample Type:	Gender:	Age Range:	Ref. Range: Critical Range: Unit:

Calibration Setup Parameters

Chem:	TRIG				
Calibration Setting	Math Model: Two-Point Linear	Calibrator	Conc.	Pos	Lot No.
Factor: Replicates: 2		Water	0.0	W	
Acceptance Limits		Chem Cal	*	*	
Cal Time: 336 hr.					
Slope Diff:	SD:				
Sensitivity:	Repeatability:				* User Defined
Deter Coeff:					
Auto Calib.					
	<input type="checkbox"/> Cal Time				

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

Calculation (Example)

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

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

Example:

$$\text{Abs Unk} = 0.243$$

$$\text{Abs Std} = 0.310$$

$$\text{Conc. Std} = 200 \text{ mg/dl}$$

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

$$\text{Triglycerides} = 157 \text{ mg/dl}$$

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

- 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.
- Comparison: A comparison was made between the Yumizen 200 series analyzers and the similar analyzer using this method on 32 samples. The correlation coefficient was 0.997. Linear regression analysis gave the following equation: This method $y = 1.031x - 1.5$.
- Precision: Precision studies were performed using the Yumizen 200 series analyzers 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.%
68.8	1.6	2.3	72.3	1.7	2.4
134.7	2.6	1.9	133.4	3.7	2.8

- 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

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

Symbol Key

Use by (YYYY-MM-DD)	Lot and batch code
Catalog number	Manufacturer
In vitro diagnostic medical device	Temperature limitation
Consult instructions for use	Rx Only: Prescription Use Only
CE mark	Authorized representative in the European Community

12-T7532-160 Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188

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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.