TRIGL

Triglycerides

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• Indicates **cobas c** systems on which reagents can be used

Order information

Triglycerides			Roche/Hitachi **cobas c** systems	
			cobas c 311	**cobas c** 501
250 tests	Cat. No. **20767107** 322	System-ID 07 6710 7	•	•
Calibrator f.a.s. (12 x 3 mL)	Cat. No. **10759350** 190	Code 401		
Calibrator f.a.s. (12 x 3 mL, for USA)	Cat. No. **10759350** 360	Code 401		
Precinorm U plus (10 x 3 mL)	Cat. No. **12149435** 122	Code 300		
Precinorm U plus (10 x 3 mL, for USA)	Cat. No. **12149435** 160	Code 300		
Precipath U plus (10 x 3 mL)	Cat. No. **12149443** 122	Code 301		
Precipath U plus (10 x 3 mL, for USA)	Cat. No. **12149443** 160	Code 301		
Precinorm U (20 x 5 mL)	Cat. No. **10171743** 122	Code 300		
Precipath U (20 x 5 mL)	Cat. No. **10171778** 122	Code 301		
Precinorm L (4 x 3 mL)	Cat. No. **10781827** 122	Code 304		
Precipath L (4 x 3 mL)	Cat. No. **11285874** 122	Code 305		
Diluent NaCl 9 % (50 mL)	Cat. No. **04489357** 190	System-ID 07 6869 3		

English

System information
TRIGL: ACN 781

Intended use
In vitro test for the quantitative determination of triglycerides in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6]
Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.

The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase. Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

Test principle[6]
Enzymatic colorimetric test.

$$\text{triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{glycerol} + 3\ \text{RCOOH}$$

$$\text{glycerol} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2\ H_2O + HCl$$

Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg^{2+}: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerokinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
Ready for use.

Storage and stability

TRIGL	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	8 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection containers.
Only the specimens listed below were tested and found acceptable.

Serum.
Plasma: Li-heparin and K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7] 5-7 days at 2-8 °C
3 months at (-15)-(-25) °C
several years at (-60)-(-80) °C

Materials provided
See "Reagents - working solutions" section for reagents.

Materials required (but not provided)
See "Order information" section.
Distilled water
General laboratory equipment

Assay
For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
The performance of applications not validated by Roche is not warranted and must be defined by the user.

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Application for serum and plasma

cobas c 311 **test definition**

Assay type	1 Point
Reaction time / Assay points	10/57
Wavelength (sub/main)	700/505 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, g/L)

Reagent pipetting		Diluent (H_2O)	
R1	120 µL	28 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501 **test definition**

Assay type	1 Point
Reaction time / Assay points	10/70
Wavelength (sub/main)	700/505 nm
Reaction direction	Increase
Units	mmol/L (mg/dL, g/L)

Reagent pipetting		Diluent (H_2O)	
R1	120 µL	28 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - and as required following quality control procedures

Traceability: This method has been standardized against the ID/MS method.

Quality control

For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors: mmol/L x 88.5 = mg/dL
mg/dL x 0.0113 = mmol/L

Limitations - interference[8]

Criterion: Recovery within ±10 % of initial values at triglyceride levels of 2.3 mmol/L (203 mg/dL).

Icterus: No significant interference up to an I index of 10 for conjugated and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L (10 mg/dL) and approximate unconjugated bilirubin concentration: 599 µmol/L (35 mg/dL)).

Hemolysis: No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L (700 mg/dL)).

Lipemia: The L index correlates with sample turbidity but not with triglycerides level. Extremely lipemic samples (triglycerides greater than 3000 mg/dL) can produce normal results.[9]

Prozone Check: The flag >Kin is an indicator for extremely high triglyceride concentrations in the sample. False normal results are due to oxygen depletion during assay reaction.

Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]
Exception: Ascorbic acid and calcium dobesilate cause artificially low triglyceride results. Intralipid is directly measured as analyte in this assay and leads to high triglyceride results.

In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. Refer to the latest version of the Carry over evasion list found with the NaOHD/SMS/Multiclean/SCCS Method Sheet and the operator manual for further instructions.
Where required, special wash/carry over evasion programming must be implemented prior to reporting results with this test.

Measuring range

0.1-10.0 mmol/L (8.85-885 mg/dL)

Determine samples having higher concentrations via the rerun function. Recommended dilution of samples via the rerun functions is a 1:5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 5.

Lower detection limit

0.1 mmol/L (8.85 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, within-run precision, n = 21).

Expected values according to NCEP[12]

Normal range: < 2.26 mmol/L (< 200 mg/dL)

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[13]

	mmol/L	mg/dL	Lipid metabolism disorder
Cholesterol Triglycerides	< 5.18 < 2.26	< 200 < 200	No
Cholesterol	5.18-7.77	200-300	Yes if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol Triglycerides	> 7.77 > 2.26	> 300 > 200	Yes

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

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Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, total n = 63).
The following results were obtained:

Within-run	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.41 (125)	0.01 (1)	0.9
Precipath U	2.40 (212)	0.02 (2)	0.8
Human serum 1	1.67 (148)	0.02 (2)	1.1
Human serum 2	2.72 (241)	0.02 (2)	0.7

Total	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.39 (123)	0.03 (3)	2.0
Precipath U	2.33 (206)	0.04 (4)	1.6
Human serum 3	1.18 (104)	0.02 (2)	1.9
Human serum 4	2.95 (261)	0.05 (4)	1.8

Method comparison

Triglycerides values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 71

Passing/Bablok[14]	Linear regression
y = 1.015x - 0.005 mmol/L	y = 1.001x + 0.018 mmol/L
τ = 0.976	r = 0.999

The sample concentrations were between 0.56 and 9.13 mmol/L (49.6 and 808 mg/dL).

References

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