04773187001V11.0

TRIGL

cobas®

Triglycerides

Order information

REF	CONTENT		Analyzer(s) on which kit(s) can be used
04657594 190	Triglycerides (4 × 50 tests)		**cobas c** 111

Materials required (but not provided):

10759350 190	Calibrator f.a.s. (12 × 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 × 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 × 3 mL)	Code 301	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 × 5 mL)	Code 300	
10171735 122	Precinorm U (4 × 5 mL)	Code 300	
10171778 122	Precipath U (20 × 5 mL)	Code 301	
10171760 122	Precipath U (4 × 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	Code 392	

English

System information

TRIGL: ACN 781

Intended use

In vitro test for the quantitative determination of triglycerides in human serum and plasma on the **cobas c** 111 system.

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

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

$$\text{glycerol} + \text{ATP} \xrightarrow[\text{MG++}]{\text{GK,}} \text{glycerol-3-phosphate} + \text{ADP}$$

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

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

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; LPL (Pseudomonas spec.): ≥ 83 µkat/L; GK (Bacillus stearothermophilus): ≥ 3 µkat/L; GPO (E. coli): ≥ 41 µkat/L; POD (horseradish): ≥ 1.6 µkat/L; preservative; stabilizers

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

H412 Harmful to aquatic life with long lasting effects.

Prevention:

P273 Avoid release to the environment.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Product safety labeling follows EU GHS guidance.

Reagent handling

Ready for use

Inaccurate pipetting of reagent, leading to potentially erroneous results, may be caused by excessive foaming of this reagent. Ensure that the foam is removed from the surface of the reagent prior to setting the reagent in the analyzer.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on reagent
On-board in use and refrigerated on the analyzer:	2 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, K_3-EDTA plasma.

EDTA tubes that are less than 1/2 full may cause a negative bias for triglycerides results.

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.

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 in serum:	2 days at 20-25 °C[7]
	10 days at 4 °C[8]
	3 months at –20 °C[9]
	several years at –70 °C[9]
Stability in plasma:	2 days at 20-25 °C[7]
	15 days at 4 °C[10]
	3 months at –20 °C[9]
	several years at –70 °C[9]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

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.

Application for serum and plasma

cobas c 111 **test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	512/659 nm
Calc. first/last	6/21
Unit	mmol/L
Reaction mode	R-S

Pipetting parameters

		Diluent (H_2O)
R	120 µL	
Sample	2 µL	28 µL
Total volume	150 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Deionized water is used automatically by the instrument as the zero calibrator.
Calibration mode	Linear regression
Calibration interval	Each lot and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the ID/MS[a)] method.

a) Isotope Dilution Mass Spectrometry

Quality control

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

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 defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The **cobas c** 111 analyzer automatically calculates the analyte concentration of each sample.

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

Note

If the free glycerol is to be taken into account, then 0.11 mmol/L (10 mg/dL) must be subtracted from the triglycerides value obtained.[9]

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at triglycerides levels of < 2.3 mmol/L (< 200 mg/dL).

Icterus:[11] No significant interference up to an I index of 5 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 57 µmol/L or 5 mg/dL).

Hemolysis:[11] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL). Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13] Exceptions: Ascorbic acid and calcium dobesilate cause artificially low triglycerides results at the tested drug levels, levodopa, methyldopa and phenylbutazone cause artificially low triglycerides results at a higher drug level and Intralipid causes artificially high triglycerides results at a higher drug level. Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[14]

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at a plasma concentration above 333 mg/L and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results. A significant interference may occur at plasma Metamizole concentrations above 0.05 mg/mL.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[15]

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 the **cobas c** 111 analyzer. For information about test combinations requiring special wash steps, please refer to the latest version of the carry over evasion list found with the CLEAN Method Sheet and the operator's manual for further instructions.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.1-10 mmol/L (8.85-885 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Lower detection limit of the test:
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 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

According to **NCEP**[16]
Normal range: < 1.70 mmol/L (< 150 mg/dL)

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[17]

	mmol/L	mg/dL	Lipid metabolism disorder
Cholesterol	< 5.2	< 200	No
Triglycerides	< 2.3	< 200	
Cholesterol	5.2-7.8	200-300	Yes if HDL-cholesterol < 0.9 mmol/L (< 35 mg/dL)
Cholesterol	> 7.8	> 300	Yes
Triglycerides	> 2.3	> 200	

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 **cobas c** 111 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 10 days). The following results were obtained:

Repeatability	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.3 (115)	0.01 (1)	0.6
Precipath U	2.2 (195)	0.02 (1)	0.8
Human serum 1	1.7 (151)	0.02 (2)	1.3
Human serum 2	5.9 (522)	0.05 (4)	0.8

Intermediate precision	*Mean mmol/L (mg/dL)*	*SD mmol/L (mg/dL)*	*CV %*
Precinorm U	1.36 (120)	0.02 (2)	1.6
Precipath U	2.34 (207)	0.04 (4)	1.7
Human serum 3	1.22 (108)	0.01 (1)	0.9
Human serum 4	8.64 (765)	0.31 (28)	3.6

Method comparison

Triglycerides values for human serum and plasma samples obtained on the **cobas c** 111 analyzer (y) were compared with those determined using the same reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 73

Passing/Bablok[18]	Linear regression
y = 1.035x – 0.017 mmol/L	y = 1.040x – 0.015 mmol/L
τ = 0.976	r = 0.998

The sample concentrations were between 0.4 and 10 mmol/L (35 and 885 mg/dL).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 Eggstein M, Kreutz FH. A new determination of the neutral fats in blood serum and tissue. I. Principles, procedure, and discussion of the method. Klin Wschr 1966;44(5):262-267.

3 Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19(5):476-482.

4 Wahlefeld AW, Bergmeyer HU, eds. Methods of Enzymatic Analysis. 2nd English ed. New York, NY:Academic Press Inc 1974;1831.

5 Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-27.

6 Siedel J, Schmuck R, Staepels J, et al. Long term stable, liquid ready-to-use monoreagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP method). AACC Meeting Abstract 34. Clin Chem 1993;39:1127.

7 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2: Jan 2002.

8 Evans K, Mitcheson J, Laker M. Effect of Storage at 4 °C and -20 °C on Lipid, Lipoprotein, and Apolipoprotein Concentrations. Clin Chem. 1995;41:392-396.

9 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;610-611.

10 Kronenberg F, Lobentanz EM, König P, et al. Effect of sample storage on the measurement of lipoprotein[a], apolipoproteins B and A-IV, total and high density lipoprotein cholesterol and triglycerides. J Lipid Res. 1994 Jul;35(7):1318-28.

11 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

12 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

13 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

14 Dastych M, Wiewiorka O, Benovska M. Ethamsylate (Dicynone) Interference in Determination of Serum Creatinine, Uric Acid, Triglycerides, and Cholesterol in Assays Involving the Trinder Reaction; In Vivo and In Vitro. Clin Lab 2014;60:1373-1376.

15 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

16 U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service, NIH Publication No. 01-3305, May 2001.

17 Study Group, European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: A policy statement of the European Atherosclerosis Society. European Heart Journal 1987;8:77-88.

18 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

TRIGL

Triglycerides

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
REAGENT	Reagent
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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