TRIGL

cobas®

Triglycerides

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058687190	08058687500	Triglycerides (1000 tests)	System-ID 2113 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information
TRIGL: ACN 21130

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

Summary
Triglyceride measurements, performed with this assay in human serum and plasma are used as an aid in identifying patients at risk of developing atherosclerosis and for the diagnosis of dyslipidemias.

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids.[1] They are partly synthesized in the liver and partly ingested in food. Triglycerides are water-insoluble molecules and are carried in the circulation in water-soluble complexes called lipoproteins. The plasma triglyceride level reflects the concentration of the triglyceride-carrying lipoproteins VLDL (very-low-density lipoproteins) and chylomicrons.[2] Chylomicrons are primarily involved in the absorption and delivery of dietary fat while VLDLs deliver endogenous lipids to other tissues.

Triglycerides are considered a risk factor for atherosclerotic cardiovascular disease.[3] Cardiovascular risk is increased when fasting triglycerides are > 1.7 mmol/L (> 150 mg/dL). Individuals with triglycerides > 2.3 mmol/L (> 200 mg/dL) are considered at high risk. The determination of triglycerides is utilized in the diagnosis of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.[4]

Elevated levels of plasma triglycerides are also associated with an increased risk of acute pancreatitis and aortic valve stenosis.[5]

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.[6] 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.[7, 8]

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.[9,10]

Test principle[10]
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; glycerol kinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative, stabilizers

R1 is in position B.

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.

Reagent handling
Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 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.

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See the limitations and interferences section for details about possible sample interferences.

Stability in serum:	2 days at 20-25 °C[11]
	10 days at 2-8 °C[12]
	3 months at -20 °C (± 5 °C)[13]
	several years at -70 °C (± 5 °C)[13]
Freeze only once.	
Stability in plasma:	2 days at 20-25 °C[11]
	15 days at 2-8 °C[14]
	3 months at -20 °C (± 5 °C)[13]
	several years at -70 °C (± 5 °C)[13]
Freeze only once.	

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

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/505 nm	
Reagent pipetting		Diluent (H_2O)
R1	66 µL	15 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.1 µL	–	–
Decreased	1.1 µL	15 µL	60 µL
Increased	1.1 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 8 weeks on-board - 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 method.

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L x 88.5 = mg/dL
	mmol/L x 0.885 = g/L

Limitations - interference

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

Icterus:[15] No significant interference up to an I index of 10 for conjugated bilirubin and 10 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L or 10 mg/dL; approximate unconjugated bilirubin concentration: 171 µmol/L or 10 mg/dL).

Hemolysis:[15] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L or 700 mg/dL).

Lipemia:[15] 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[16].

Prozone Check: The flag > Kin is an indicator for extremely high triglyceride concentrations in the sample. False low 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.[17,18]

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.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[19]

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at a plasma concentration above 166 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.[20]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.1-10.0 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 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

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Limit of Blank	= 0.1 mmol/L (8.85 mg/dL)
Limit of Detection	= 0.1 mmol/L (8.85 mg/dL)
Limit of Quantitation	= 0.1 mmol/L (8.85 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration triglycerides samples.

Expected values according to NCEP[21]

mmol/L

Normal range: < 1.70 mmol/L

Clinical interpretation according to the recommendations of the European Atherosclerosis Society: [22]

	mmol/L	Lipid metabolism disorder
Cholesterol Triglycerides	< 5.18 < 2.26	No
Cholesterol	5.18-7.77	Yes if HDL-cholesterol < 0.9 mmol/L
Cholesterol Triglycerides	> 7.77 > 2.26	Yes

mg/dL

Normal range: < 150 mg/dL

Clinical interpretation according to the recommendations of the European Atherosclerosis Society: [22]

	mg/dL	Lipid metabolism disorder
Cholesterol Triglycerides	< 200 < 200	No
Cholesterol	200-300	Yes if HDL-cholesterol < 35 mg/dL
Cholesterol Triglycerides	> 300 > 200	Yes

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.[13]

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	1.37	0.00824	0.6
PCCC2[b)]	2.50	0.0150	0.6
Human serum 1	0.195	0.00414	2.1
Human serum 2	1.73	0.0107	0.6
Human serum 3	3.14	0.0229	0.7
Human serum 4	5.25	0.0324	0.6
Human serum 5	8.56	0.0476	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	1.37	0.0104	0.8
PCCC2[b)]	2.51	0.0209	0.8
Human serum 1	0.195	0.00443	2.3
Human serum 2	1.73	0.0126	0.7
Human serum 3	3.14	0.0250	0.8
Human serum 4	5.23	0.0350	0.7
Human serum 5	8.50	0.0555	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Triglycerides values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[23]	Linear regression
y = 1.015x + 0.0125 mmol/L	y = 1.020x + 0.00786 mmol/L
τ = 0.983	r = 0.999

The sample concentrations were between 0.300 and 9.19 mmol/L.

Triglycerides values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[23]	Linear regression
y = 1.019x - 0.00772 mmol/L	y = 1.021x - 0.0114 mmol/L
τ = 0.994	r = 1.000

The sample concentrations were between 0.170 and 9.63 mmol/L.

Triglycerides values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

Passing/Bablok[23]	Linear regression
y = 1.018x - 0.0236 mmol/L	y = 1.022x - 0.0320 mmol/L
τ = 0.995	r = 1.000

The sample concentrations were between 0.611 and 9.72 mmol/L.

References

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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 navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606