

## Triglycerides

### Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
08058687190	Triglycerides (1000 tests)	System-ID 2113 001 <b>cobas c 303, cobas c 503</b>
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 Roche/Hitachi **cobas c** systems.

#### Summary<sup>1,2,3,4,5,6</sup>

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.

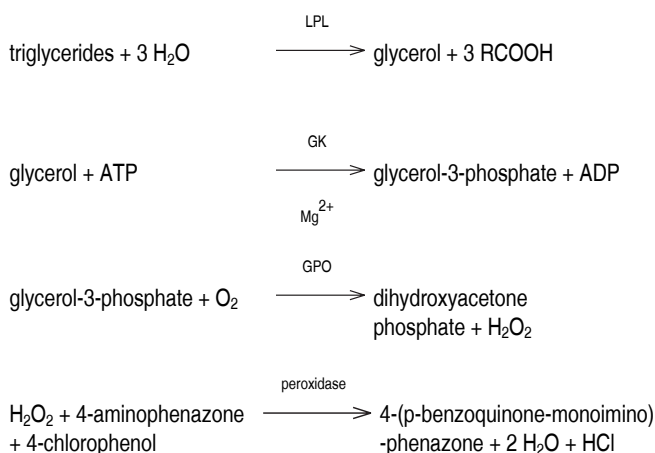
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

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<sup>6</sup>

Enzymatic colorimetric test.



### Reagents - working solutions

**R1** PIPES buffer: 50 mmol/L, pH 6.8; Mg<sup>2+</sup>: 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.

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.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

#### 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<sub>2</sub>-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

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tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. See the limitations and interferences section for details about possible sample interferences.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Stability in serum:	2 days at 20-25 °C <sup>7</sup>
	10 days at 4 °C <sup>8</sup>
	3 months at -20 °C <sup>9</sup>
	several years at -70 °C <sup>9</sup>
Stability in plasma:	2 days at 20-25 °C <sup>7</sup>
	15 days at 4 °C <sup>10</sup>
	3 months at -20 °C <sup>9</sup>
	several years at -70 °C <sup>9</sup>

### 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 <sub>2</sub> O)	
R1	66 µL	15 µL	
<b>Sample volumes</b>			
	<i>Sample</i>	<i>Sample dilution</i>	
		<i>Sample</i>	<i>Diluent (NaCl)</i>
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 <sub>2</sub> O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration
	- after reagent lot change
	- 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:<sup>11</sup> No significant interference up to an I index of 10 for conjugated bilirubin and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L or 10 mg/dL; approximate unconjugated bilirubin concentration: 599 µmol/L or 35 mg/dL).

Hemolysis:<sup>11</sup> No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L or 700 mg/dL).

Lipemia:<sup>11</sup> 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<sup>12</sup>.

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.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.<sup>13</sup>

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>14,15</sup>

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.

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.<sup>16</sup>

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 information. For further instructions refer to the operator's manual.

### Limits and ranges

#### Measuring range

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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*

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 95<sup>th</sup> percentile value from  $n \geq 60$  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<sup>17</sup>

mmol/L

Normal range: < 1.70 mmol/L

**Clinical interpretation** according to the recommendations of the European Atherosclerosis Society: <sup>18</sup>

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

mg/dL

Normal range: < 150 mg/dL

**Clinical interpretation** according to the recommendations of the European Atherosclerosis Society: <sup>18</sup>

	mg/dL	Lipid metabolism disorder
Cholesterol	< 200	No
Triglycerides	< 200	No
Cholesterol	200-300	Yes if HDL-cholesterol < 35 mg/dL
Cholesterol	> 300	Yes
Triglycerides	> 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.<sup>9</sup>

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 heterogeneous 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 <sup>a)</sup>	1.37	0.00824	0.6
PCCC2 <sup>b)</sup>	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 <sup>a)</sup>	1.37	0.0104	0.8
PCCC2 <sup>b)</sup>	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).

### 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 <sup>19</sup>	Linear regression
$y = 1.015x + 0.0125$ mmol/L	$y = 1.020x + 0.00786$ mmol/L
$r = 0.983$	$r = 0.999$

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 <sup>19</sup>	Linear regression
$y = 1.019x - 0.00772$ mmol/L	$y = 1.021x - 0.0114$ mmol/L
$r = 0.994$	$r = 1.000$

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

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Additions, deletions or changes are indicated by a change bar in the margin.

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**CE** 0123



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

**CONTENT**

Contents of kit



Volume after reconstitution or mixing

**GTIN**

Global Trade Item Number

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