

## Triglycerides

### Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20767107 322	Triglycerides 250 tests	System-ID 07 6710 7 <b>cobas c 311, cobas c 501/502</b>
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401
10171743 122	Precinorm U (20 x 5 mL)	Code 300
10171735 122	Precinorm U (4 x 5 mL)	Code 300
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300
10781827 122	Precinorm L (4 x 3 mL)	Code 304
10171778 122	Precipath U (20 x 5 mL)	Code 301
10171760 122	Precipath U (4 x 5 mL)	Code 301
12149443 122	Precipath U plus (10 x 3 mL)	Code 301
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301
11285874 122	Precipath L (4 x 3 mL)	Code 305
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3

### English

#### System information

For **cobas c 311/501** analyzers:

**TRIGL**: ACN 781

For **cobas c 502** analyzer:

**TRIGL**: ACN 8781

#### 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.

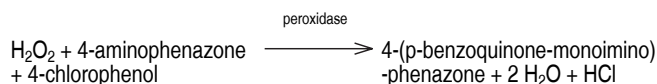
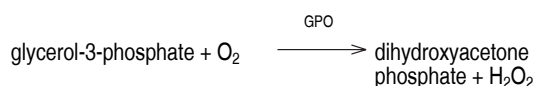
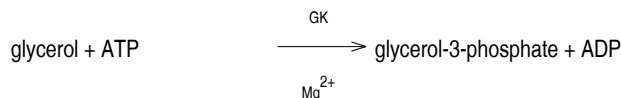
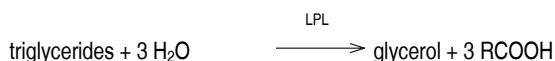
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

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;  $\text{Mg}^{2+}$ : 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP:  $\geq 1.4$  mmol/L; 4-aminophenazone:  $\geq 0.13$  mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (*Pseudomonas spec.*):  $\geq 83$   $\mu\text{kat/L}$ ; glycerokinase (*Bacillus stearothermophilus*):  $\geq 3$   $\mu\text{kat/L}$ ; glycerol phosphate oxidase (*E. coli*):  $\geq 41$   $\mu\text{kat/L}$ ; peroxidase (horseradish):  $\geq 1.6$   $\mu\text{kat/L}$ ; preservative, stabilizers

R1 is in position B.

#### Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

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

Centrifuge samples containing precipitates before performing the assay.

Stability:<sup>7</sup> 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

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 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 <sub>2</sub> O)		
R1	120 µL	28 µL	
<b>Sample volumes</b>	<b>Sample</b>	<b>Sample dilution</b>	
		<b>Sample</b>	<b>Diluent (NaCl)</b>
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	2 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 <sub>2</sub> O)
R1	120 µL 28 µL

<b>Sample volumes</b>	<b>Sample</b>	<b>Sample dilution</b>	
		<b>Sample</b>	<b>Diluent (NaCl)</b>
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	2 µL	–	–

**cobas c 502 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 <sub>2</sub> O)
R1	120 µL 28 µL

<b>Sample volumes</b>	<b>Sample</b>	<b>Sample dilution</b>	
		<b>Sample</b>	<b>Diluent (NaCl)</b>
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	4 µL	–	–

**Calibration**

Calibrators S1: H<sub>2</sub>O  
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.

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**

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

Conversion factors:

mmol/L x 88.5 = mg/dL	mmol/L x 0.885 = g/L
mg/dL x 0.0113 = mmol/L	mg/dL x 0.01 = g/L

## Limitations - interference

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

Icterus:<sup>8</sup> No significant interference up to an I index of 10 for conjugated bilirubin and 35 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171  $\mu\text{mol/L}$  or 10 mg/dL; approximate unconjugated bilirubin concentration: 599  $\mu\text{mol/L}$  or 35 mg/dL).

Hemolysis:<sup>8</sup> No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434  $\mu\text{mol/L}$  or 700 mg/dL).

Lipemia:<sup>9</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>9</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.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>10,11</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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>12</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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOH/SMS/Multiclean/SCCS or the NaOH/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

## Limits and ranges

### Measuring range

0.1-10.0 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

*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<sup>13</sup>

Normal range: < 1.70 mmol/L (< 150 mg/dL).

**Clinical interpretation** according to the recommendations of the European Atherosclerosis Society:<sup>14</sup>

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

Cholesterol	> 7.77	> 300	Yes
Triglycerides	> 2.26	> 200	

**Note:** If the free glycerol is to be taken into account, the 0.11 mmol/L (10 mg/dL) must be subtracted from the triglycerides value obtained.<sup>7</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. 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, 21 days). The following results were obtained:

	Repeatability		
	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

	Intermediate precision		
	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 corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 71

Passing/Bablok <sup>15</sup>	Linear regression
$y = 1.015x - 0.005 \text{ mmol/L}$	$y = 1.001x + 0.018 \text{ mmol/L}$
$r = 0.976$	$r = 0.999$

The sample concentrations were between 0.560 and 9.13 mmol/L (49.6 and 808 mg/dL).

## References

- Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.
- 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.
- Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19(5):476-482.
- Wahlefeld AW, Bergmeyer HU, eds. Methods of Enzymatic Analysis. 2nd English ed. New York, NY: Academic Press Inc 1974;1831.
- Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24-27.
- 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.
- Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;610-611.

# TRIGL

## Triglycerides



- 8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 9 Shephard MDS, Whiting MJ. Falsely low estimation of triglycerides in lipemic plasma by the enzymatic triglyceride method with modified Trinder's chromogen. Clin Chem 1990;36(2):325-329.
- 10 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 11 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.
- 12 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 13 U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service, NIH Publication No. 01-3305, May 2001.
- 14 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.
- 15 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.

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.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT

Contents of kit



Volume after reconstitution or mixing

### 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.

COBAS, COBAS C, PRECICONTROL, PRECINORM and PRECIPATH are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Significant additions or changes are indicated by a change bar in the margin.

© 2013, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
www.roche.com

Distribution in USA by:

Roche Diagnostics, Indianapolis, IN

US Customer Technical Support 1-800-428-2336

