0108057796190c503V4.0

GGT-2

cobas®

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** packs can be used
08057796190	γ-Glutamyltransferase ver.2 (400 tests)	System-ID 2060 001	**cobas c** 303, **cobas c** 503

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

GGT2-I: ACN 20600: assay standardized against IFCC

GGT2-S: ACN 20601: assay standardized against Szasz

Intended use

In vitro test for the quantitative determination of γ-glutamyltransferase (GGT) in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6]

γ-glutamyltransferase is used in the diagnosis and monitoring of hepatobiliary diseases. Enzymatic activity of GGT is often the only parameter with increased values when testing for such diseases, and is one of the most sensitive indicators known. γ-glutamyltransferase is also a sensitive screening test for occult alcoholism. Elevated GGT activities are found in the serum of patients requiring long-term medication with phenobarbital and phenytoin.

In 1969, Szasz published the first kinetic procedure for GGT in serum using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor. In order to circumvent the poor solubility of γ-glutamyl-p-nitroanilide, Persijn and van der Slik investigated various derivatives and found the water-soluble substrate L-γ-glutamyl-3-carboxy-4-nitroanilide to be superior in terms of stability and solubility. The results correlate with those derived using the original substrate.

In 2002, the International Federation of Clinical Chemistry (IFCC) recommended the standardized method for determining GGT including optimization of substrate concentrations, employment of NaOH, glycylglycine buffer and sample start. The GGT liquid reagent follows the formulation recommendation according to Szasz, but was optimized for performance and stability. The assay is optionally standardized against the original IFCC and Szasz methods. The performance claims and data presented here are independent from the standardization.

Test principle[7]

Enzymatic colorimetric assay

γ-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{GGT}}$ L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoate

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance photometrically.

Reagents - working solutions

R1 TRIS: 492 mmol/L, pH 8.25; glycylglycine: 492 mmol/L; preservative; additive

R3 L-γ-glutamyl-3-carboxy-4-nitroanilide: 22.5 mmol/L; acetate: 10 mmol/L, pH 4.5; stabilizer; preservative

R1 is in position B and R3 is in position C.

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:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

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:	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: Collect serum using standard sampling tubes.
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.

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

Stability:[8,9]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at (-15)-(-25) °C

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.

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)	700/415 nm		
Reagent pipetting		Diluent (H_2O)	
R1	19 µL	57 µL	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	–	–
Decreased	2.3 µL	10 µL	100 µL
Increased	2.3 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 - as required following quality control procedures

Use the appropriate calibrator value for the corresponding application.

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

Traceability: This method has been standardized against the original IFCC formulation (2002)[5] and against the GGT method published by Persijn and van der Slik (1976)[4], respectively.

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 12 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interferences

Criterion: Recovery within ± 10 % of initial value at a γ-glutamyltransferase activity of 40 U/L.

Icterus:[10] No significant interference up to an I index of 50 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 855 µmol/L or 50 mg/dL and approximate unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis:[10] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[10] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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

3-1200 U/L (0.05-20.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 11.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 3 U/L (0.05 µkat/L)

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 activity 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 activity samples.

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

GGT-2

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

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

Expected values

U/L

Standardized against Szasz (Persijn, van der Slik)[14]

Men	8-61 U/L
Women	5-36 U/L

Standardized against IFCC

Reference Interval Study at 37 °C (corrected in 2005)[14,15]

Men (n = 216)	10-71 U/L
Women (n = 228)	6-42 U/L

Consensus values (IFCC)[16]

Men	< 60 U/L
Women	< 40 U/L

µkat/L

Standardized against Szasz (Persijn, van der Slik)[14,*]

Men	0.13-1.02 µkat/L
Women	0.08-0.60 µkat/L

Standardized against IFCC

Reference Interval Study at 37 °C (corrected in 2005)[14,15,*]

Men (n = 216)	0.17-1.19 µkat/L
Women (n = 228)	0.10-0.70 µkat/L

Consensus values (IFCC)[16]

Men	< 1.00 µkat/L
Women	< 0.67 µkat/L*

*calculated by unit conversion factor

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* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	45.8	0.382	0.8
PCCC2[b)]	207	0.772	0.4
Human serum 1	8.57	0.449	5.2
Human serum 2	30.9	0.646	2.1
Human serum 3	62.7	0.679	1.1
Human serum 4	598	3.55	0.6
Human serum 5	1155	6.04	0.5

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	45.6	0.463	1.0
PCCC2[b)]	207	1.67	0.8
Human serum 1	7.97	0.420	5.3
Human serum 2	30.6	0.703	2.3
Human serum 3	62.7	0.708	1.1
Human serum 4	598	3.69	0.6
Human serum 5	1161	10.6	0.9

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

γ-glutamyltransferase 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) = 65

Passing/Bablok[17]	Linear regression
y = 1.014x – 1.98 U/L	y = 1.023x – 1.96 U/L
τ = 0.981	r = 0.999

The sample activities were between 4.81 and 941 U/L.

γ-glutamyltransferase 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) = 75

Passing/Bablok[17]	Linear regression
y = 1.010x + 1.44 U/L	y = 1.019x + 0.534 U/L
τ = 0.982	r = 1.000

The sample activities were between 3.10 and 1001 U/L.

References

1 Thomas L, ed. Labor und Diagnose, 4th ed. Marburg: Die Medizinische Verlagsgesellschaft 1992.

2 Shaw LM. Keeping pace with a popular enzyme GGT. Diagnostic Medicine May/June 1982;1–8.

3 Szasz G. A kinetic photometric method for serum γ-glutamyl-transferase. J Clin Chem 1969;15:124-136.

4 Persijn JP, van der Slik W. A new Method for the Determination of γ-Glutamyltransferase. J Clin Chem Clin Biochem 1976;4:421.

5 Schumann G, Bonora R, Ceriottiet F et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 6. Reference Procedure for the Measurement of Catalytic Activity Concentrations of gamma-glutamyltransferase. Clin Chem Lab Med 2002;40(7):734-738.

6 Klauke R, Schmidt E, Lorentz K. Recommendations for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase at 37 °C. Eur J Clin Chem Clin Biochem 1993;31:901-909.

7 Szasz G, Weimann G, Stähler F, et al. New Substrates for measuring gamma-glutamyl-transpeptidase activity. Z Klin Chem Klin Biochem 1974;12:228-233.

8 Szasz G. Methods of Enzymatic Analysis. 2nd English ed. New York. Academic Press, Inc 1974;717.

9 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;286.

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

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

12 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.

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

14 Abicht K, El-Samalouti V, Junge W, et al. Multicenter evaluation of new GGT and ALP reagents with new reference standardization and determination of 37 °C reference intervals. Clin Chem Lab Med 2001;39:Special Supplement pp S 346.

15 Kytzia H-J. Reference intervals for GGT according to the new IFCC 37°C reference procedure. Clin Chem Lab Med 2005;43:A69 [abstract].

16 Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29(5):301-308.

17 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.

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

CE 0123

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