CARB4

cobas®

ONLINE TDM Carbamazepine Gen.4

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
07258062 190	ONLINE TDM Carbamazepine Gen.4 (100 tests)	System-ID 07 7587 8	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03375790 190	Preciset TDM I Calibrators B-F (5 × 1 × 5 mL) Diluent (1 × 10 mL)	Codes 692-696	
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	 Code 310 Code 311 Code 312	

English

System information

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

CARB4: ACN 144

For **cobas c** 502 analyzer:

CARB4: ACN 8144

Intended use

In vitro test for the quantitative determination of carbamazepine in serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Carbamazepine is an anticonvulsant drug, used in particular for the treatment of trigeminal neuralgia,[1] all forms of partial epilepsy, generalized tonic-clonic seizures, and simple and complex partial seizures.[2,3,4] The specific mechanism of carbamazepine is proposed as a depressant action on transmission through the nucleus ventralis anterior of the thalamus.[2,3] Carbamazepine, 5H-dibenz[b,f]-azepine-5-carboxamide, is an iminostilbene derivative also recognized by its common brand name, Tegretol. In the circulation, carbamazepine is approximately 70 % bound by protein.[3,4,5] The drug is metabolized to carbamazepine-10,11-epoxide, which is pharmacologically active, and then to carbamazepine-10,11-dihydroxide, both of which are excreted in urine. The plasma concentration of the epoxide metabolite ranges from 15 % to 48 % of the parent compound.[6] The epoxide has a shorter half-life (5-8 hours) than the parent compound (8-60 hours).[2,3,4] The epoxide and the 10,11-dihydroxide are excreted unaltered or after conjugation to glucuronic acid.

In combination with other clinical information, monitoring carbamazepine levels provides physicians with an effective tool to aid in adjusting dosage and achieving optimal therapeutic effect while avoiding both subtherapeutic and toxic drug levels.

Test principle

The ONLINE TDM Carbamazepine Gen.4 assay is a homogeneous microparticle agglutination immunoassay. It is a two-reagent system used for the detection of carbamazepine in serum. Kinetic interaction of microparticles (KIMS) will be measured using automated analyzers. In this technology biotinylated drug hapten attached to streptavidin coated latex beads serves as the binding partner to anti-carbamazepine antibody. A competitive reaction to a limited amount of specific anti-carbamazepine antibody takes place between the latex bound hapten and free carbamazepine in the serum sample. A decrease in the apparent signal is proportional to the amount of drug present in the sample.

Reagents - working solutions

R1 Anti-carbamazepine antibody (sheep monoclonal); MES[a)] buffer, pH 6.4; preservative

R2 Carbamazepine biotinylated hapten; streptavidin coated latex microparticles: 0.1 %; HEPES[b)] buffer, pH 7.4; preservative

a) 2-(N-Morpholino) ethanesulfonic acid

b) N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)

R1 is in position B and R2 is in position C.

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.

For USA: For prescription use only.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

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:	4 weeks

Do not freeze.

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: K_2- and K_3-EDTA, sodium or lithium heparin plasma.

Stability:[7]	2 days capped at 20-25 °C
	7 days capped at 2-8 °C
	4 weeks capped at –20 °C

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.

Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

Usual sampling time varies dependent upon desired measurement of peak or trough values.[8]

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

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

CARB4

ONLINE TDM Carbamazepine Gen.4

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 9-52		
Wavelength (sub/main)	800/546 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	116 µL	–	
R2	116 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	–	–
Increased	1.5 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 15-63		
Wavelength (sub/main)	800/546 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	116 µL	–	
R2	116 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	–	–
Increased	1.5 µL	–	–

Calibration

Calibrators	S2-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	5-point calibration ▪ after reagent lot change ▪ every 35 days ▪ as required following quality control procedures

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of carbamazepine in normal human serum.

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 factor:[9] µg/mL × 4.23 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at carbamazepine levels of approximately 3 and 12 µg/mL (12.7 and 50.8 µmol/L).

Serum/Plasma

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

Hemolysis:[10] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid):[10] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 1200 IU/mL.

Total protein: No significant interference from total protein up to 13 g/dL.

Cholesterol: No significant interference from cholesterol up to 600 mg/dL.

As with any assay employing sheep antibodies, the possibility exists for interference by human anti-sheep antibodies in the sample, which could cause unreliable results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

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 NaOHD-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

2-20 µg/mL (8.5-85 µmol/L)

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I Diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value. Linearity was verified for the measuring range according to CLSI EP-6 guidelines.

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.5 µg/mL (2.1 µmol/L)
Limit of Detection	= 1.0 µg/mL (4.2 µmol/L)
Limit of Quantitation	= 2.0 µg/mL (8.5 µmol/L)

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

Expected values

The therapeutic range for carbamazepine is derived from the relationships between plasma level, seizure control and emergence of side effects. Blood levels vary depending on sex, race and age. Although other ranges are also quoted, the therapeutic range is often set between 4 and 12 µg/mL (16.9-50.8 µmol/L).[12,13]

Lower concentrations may provide effective therapeutic response when other anticonvulsants are used in combination with carbamazepine.[14,15]

Serum or plasma level monitoring provides an indicator for individual dosage regimen. Some patients may require levels outside these ranges for effective treatment. The ranges are therefore, provided only as a guide for interpretation along with other clinical symptoms, and are not to be taken as the sole indicator for adjustment of dosage. Peak concentrations above 12 µg/mL (50.8 µmol/L) are often associated with toxicity.[16]

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 accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5-A2 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer:

Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
TDM Control Set Level 1	3.4	14	0.1	0.4	2.2
TDM Control Set Level 2	9.7	41	0.1	0.4	1.4
TDM Control Set Level 3	15.7	66	0.2	0.8	1.3
Human serum 1	2.9	12	0.1	0.4	2.7
Human serum 2	4.2	18	0.1	0.4	2.1
Human serum 3	9.4	40	0.2	0.8	1.8
Human serum 4	14.6	62	0.2	0.8	1.3
Human serum 5	19.5	82	0.3	1.3	1.4

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
TDM Control Set Level 1	3.4	14	0.1	0.4	2.8
TDM Control Set Level 2	9.7	41	0.2	0.8	2.3
TDM Control Set Level 3	15.7	66	0.3	1.3	1.8
Human serum 1	2.9	12	0.1	0.4	3.3
Human serum 2	4.2	18	0.1	0.4	2.9
Human serum 3	9.4	40	0.3	1.3	2.6
Human serum 4	14.6	62	0.4	1.7	2.4
Human serum 5	19.5	82	0.6	2.5	2.9

Method comparison

Carbamazepine values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the Roche ONLINE TDM Carbamazepine (CARB2) reagent on a Roche/Hitachi **cobas c** 501 analyzer (x).
Sample size (n) = 100

Deming regression weighted[17]

y = 0.994x + 0.054 µg/mL

r = 0.993

The sample concentrations were between 2.38 and 18.7 µg/mL (10.1 and 79.1 µmol/L).

Carbamazepine values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using LC/MS (x).
Sample size (n) = 100

Deming regression weighted[17]

y = 0.935x + 0.466 µg/mL

r = 0.991

The sample concentrations were between 2.00 and 19.1 µg/mL (8.46 and 80.8 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity at low (3 µg/mL) and high (12 µg/mL) carbamazepine concentration.

Carbamazepine concentration 3 µg/mL:

Compound	Compound concentration µg/mL	Cross-reactivity %
Carbamazepine-10,11-epoxide	29.6	2.9
10-Hydroxycarbamazepine (MHD)	100	0.6
Oxcarbazepine (Oxc)	100	0.9
Nortriptyline	50	ND
Amitriptyline	100	ND
Imipramine	200	ND
Phenothiazine	200	ND
Phenylbutazone	450	0.1
Promethazine	1000	ND
Phenytoin	1000	ND
Mephenytoin	1000	0.1
2-Phenyl-2-ethylmalonamide	1000	0.3
Valproic Acid	1000	ND
Amobarbital	1000	ND
Chlordiazepoxide	30	0.3
Clonazepam	12	0.4
Ethosuximide	1000	ND
Ethotoin	1000	0.1
Diazepam	25	0.2
Glutethimide	1000	ND
Methosuximide	100	ND
p-Hydroxyphenobarbital	100	0.1
5-(p-Hydroxyphenyl)-phenylhydantoin	1000	ND
Phenobarbital	1000	ND
Primidone	1000	ND
Probenecid	500	ND
Secobarbital	1000	ND

ND = Not Detected

Carbamazepine concentration 12 µg/mL:

Compound	Compound concentration µg/mL	Cross-reactivity %
Carbamazepine-10,11-epoxide	29.6	1.4
10-Hydroxycarbamazepine (MHD)	100	0.2
Oxcarbazepine (Oxc)	100	0.2
Nortriptyline	50	0.3
Amitriptyline	100	ND
Imipramine	200	ND

CARB4

ONLINE TDM Carbamazepine Gen.4

Compound	Compound concentration µg/mL	Cross-reactivity %
Phenothiazine	200	ND
Phenylbutazone	450	ND
Promethazine	1000	ND
Phenytoin	1000	ND
Mephenytoin	1000	0.1
2-Phenyl-2-ethylmalonamide	1000	0.2
Valproic Acid	1000	ND
Amobarbital	1000	ND
Chlordiazepoxide	30	0.4
Clonazepam	12	0.3
Ethosuximide	1000	ND
Ethotoin	1000	0.1
Diazepam	25	0.4
Glutethimide	1000	ND
Methosuximide	100	ND
p-Hydroxyphenobarbital	100	0.4
5-(p-Hydroxyphenyl)-phenylhydantoin	1000	ND
Phenobarbital	1000	ND
Primidone	1000	ND
Probenecid	500	ND
Secobarbital	1000	ND

ND = Not Detected

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H_2O
Ascorbic acid	Metronidazole
Heparin	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

References

1 Blom S. Trigeminal neuralgia: its treatment with a new anticonvulsant drug (G-32883). Lancet 1962;839-840.

2 Eadie MJ, Tyler JH. Anticonvulsant Therapy: Pharmacological Basis and Practice. Edinburgh, Great Britain: Churchill Livingstone 1974;chap 7.

3 Penry JK, Newmark ME. The use of antiepileptic drugs. Ann Int Med 1979;90:207-218.

4 Scheuer ML, Pedley TA. The evaluation and treatment of seizures. N Engl J Med 1990;322(21):1468-1474.

5 MacKichan JJ, Duffner PK, Cohen ME. Salivary concentrations and plasma protein binding of carbamazepine and carbamazepine-10,11-epoxide in epileptic patients. Eur J Clin Pharm 1981;12:31-37.

6 Morselli PL. Carbamazepine: Absorption, distribution and excretion in complex partial seizures and their treatment. In: Penry JK, Daly DD eds. Advances in Neurology, Vol II. New York, NY: Raven Press 1975:279-293.

7 Data on file at Roche Diagnostics.

8 Jacobs DS, Kaster BL Jr, Demott WR, et al. Laboratory Test Handbook. Stowe, OH. Lexi-Compl. Mosby 1990:812.

9 Tietz NW. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia, PA: WB Saunders Co 1995:834.

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

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

12 Arroyo S, Sander JWAS. Carbamazepine in comparative trials: pharmacokinetic characteristics too often forgotten. Neurology 1999;53(6):1170-1174.

13 Goldman L, Ausiello D, editors. Cecil Medicine, 23rd ed. Philapelphia PA: Elsevier Saunders; 2008: 2983-2995.

14 Shorvon SD, Chadwick D, Galbraith AW, et al. One drug for epilepsy. Br Med J 1978;1:474-476.

15 Altafullah I, Talwar D, Loewenson R, et al. Factors influencing serum levels of carbamazepine and carbamazepine-10,11-epoxide in children. Epilepsy Res 1989;4(1):72-80.

16 Pincus MR, Abraham NZ Jr. Toxicology and therapeutic drug monitoring. Henry JB (ed.): Clinical Diagnosis and Management by Laboratory Methods, ed. 18. Philadelphia, WB Saunders Co 1991:349-384.

17 Linnet, K. Evaluation of regression procedures for method comparison studies. Clinical Chemistry 1993 Mar;39(3):424-432.

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
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.

CE

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