CARB3

cobas®

Carbamazepine

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04874625 190	CEDIA Carbamazepine (125 tests)	System-ID 07 6952 5	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
11815253 216	CEDIA Core TDM Multi-Cal Low (2 x 7.5 mL) High (2 x 5 mL)	 Code 662 Code 663	
04521536 190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 310 Code 311 Code 312	

Opening tool: On request

English

System information

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

CARB3: ACN 31

For **cobas c** 502 analyzers

CARB3: ACN 8031

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[7]

The CEDIA Carbamazepine assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.

The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment of β-galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme is formed.

The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

Reagents - working solutions

R1 EA working solution
anti-carbamazepine antibody (mouse monoclonal): 49 mg/L; enzyme acceptor (microbial): 0.171 g/L; 3-(N-morpholino) propane sulfonic acid (MOPS) buffer; stabilizer; buffer salts; preservative

R3 ED working solution
enzyme donor (microbial)-carbamazepine conjugate: 22.1 µg/L; chlorophenol red-β-D-galactopyranoside: 1.64 g/L; 2-(N-morpholinio) ethane sulfonic acid (MES) buffer; buffer salts; stabilizer; preservative

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.

CAUTION. WARNING. The reagents contain less than 1 % sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10 % sodium hydroxide.

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 primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Prepare the cobas CEDIA Carbamazepine working solutions using cold reagents and buffers. Remove the kit from refrigerated storage (2-8 °C) immediately prior to preparation of the working solutions. **Prepare the solutions in the following order to minimize possible contamination:**

1. **R3:** Connect bottle R3a (ED reagent) to bottle R3 (ED Reconstitution Buffer) using one of the 4 enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from bottle R3a is transferred into bottle R3. Avoid the formation of foam. Detach bottle R3a and leave the adapter on bottle R3.

2. Insert the opening tool into the **cobas c** Carbamazepine cassette position C bottle cap until it clicks and unscrew the cap (position C = R3, also indicated on the upper part of the cassette label). It is located on the far right when looking at the main cassette label. Save the cap for recapping later in this procedure.

3. Invert the **cobas c** Carbamazepine cassette. Connect bottle R3 (and adapter) to position C of the **cobas c** Carbamazepine cassette and press down. Invert assembled containers to transfer contents of bottle R3 working solution into the **cobas c** Carbamazepine cassette.

NOTE: Ensure entire contents of bottle R3 are transferred into the cassette position C bottle.

4. Detach bottle R3 and adapter and tightly cap the **cobas c** Carbamazepine cassette using the cap saved in step 2.

5. **R1:** Connect bottle R1a (EA reagent) to bottle R1 (EA Reconstitution Buffer) using the second of the 4 enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from bottle R1a is transferred into bottle R1. Avoid the formation of foam. Detach bottle R1a and leave the adapter on bottle R1.

6. Insert the opening tool into the **cobas c** Carbamazepine cassette position B bottle cap until it clicks and unscrew the cap (position B = R1, also indicated on the upper part of the cassette label). It is located on the far left when looking at the main cassette label. Save the cap for recapping later in this procedure.

7. Invert the **cobas c** Carbamazepine cassette. Connect bottle R1 (and adapter) to position B of the **cobas c** Carbamazepine cassette and press down. Invert assembled containers to transfer contents of bottle R1 working solution into the **cobas c** Carbamazepine cassette.

NOTE: Ensure entire contents of bottle R1 are transferred into the cassette position B bottle.

8. Detach bottle R1 and adapter and tightly cap the **cobas c** Carbamazepine cassette using the cap saved in step 6.

9. Allow the **cobas c** Carbamazepine cassette to sit approximately 5 minutes at 15-25 °C. Mix by gentle inversion five times. Place the **cobas c** Carbamazepine cassette directly onto the **cobas c** system.
NOTE: the cassette must be on the analyzer 30 minutes before use.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots. Always use a new **cobas c** Carbamazepine cassette when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results.

NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R3 Working Solution (Enzyme Donor) should be yellow-orange in color. A red or purple-red color indicates that the reagent has been contaminated and must be discarded.

NOTE 3: If the **cobas c** Carbamazepine cassette is not filled correctly this may result in faulty reagent pipetting and could cause erroneous results.

NOTE 4: The R1 and R3 Working Solutions must be at the reagent compartment temperature of the analyzer before performing the assay.

Storage and stability

Unopened kit components: up to the expiration date at 2-8 °C

On-board in use and refrigerated on the analyzer: 60 days

Do not freeze.

To ensure reconstituted EA reagent stability, protect from prolonged continuous exposure to bright light.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Some gel separation tubes may not be suitable for use with therapeutic drug monitoring assays.

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.

Avoid repeated freezing and thawing. Do not induce foaming of specimens.

Invert thawed specimens several times prior to testing.

Centrifuge samples containing precipitates before performing the assay.

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes.
Plasma: Sodium and lithium heparin or K_2 and K_3-EDTA plasma.

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

For the purpose of monitoring blood levels, consistency of sample collection timing after administration of the last drug dose improves the safety and efficacy of the anticonvulsant. The timing of specimen collection can influence the relationship between carbamazepine concentration and the clinical response. Other pharmacokinetic factors such as mode of administration, dosage form, concomitant drug therapy, and biological variations in drug absorption should be taken into consideration.

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.

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 53-57		
Wavelength (sub/main)	660 / 570 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	106 µL	–	
R3	106 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

cobas c 501/502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 64-70		
Wavelength (sub/main)	660 / 570 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	106 µL	–	
R3	106 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Calibration

Calibrator	CEDIA Core TDM Multi-Cal
Calibration mode	Linear

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Calibration frequency 2-point calibration
- after **cobas c** pack change
- after reagent lot change
- 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 protein buffer matrix containing bovine serum albumin.

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 x 4.23 = µmol/L

Limitations - interference

Criterion: Recovery within ± 0.5 µg/mL (2.1 µmol/L) of initial value at concentrations < 5 µg/mL (21.2 µmol/L) or ± 10 % of initial value at concentrations > 5 µg/mL (21.2 µmol/L).

Serum/Plasma

Icterus:[10] No significant interference up to an I index of 8 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 137 µmol/L or 8 mg/dL; approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

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

Lipemia (Intralipid):[10] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to 180 IU/mL.

Total protein: No interference from total protein up to 12 g/dL.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely elevated results.

The incidence of patients with antibodies to *E. coli* β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high carbamazepine results that do not fit the clinical profile. If this occurs, contact Customer Technical Support.

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

0.5-20 µg/mL (2.1-84.6 µmol/L)

Specimen dilution

Manually dilute samples above the measuring range 1 + 1 with CEDIA Core TDM Multi-Cal Low calibrator and reassay. Multiply the result by 2 and subtract the concentration of the low calibrator to obtain the specimen value.

Lower limits of measurement

Lower detection limit of the test

0.5 µg/mL (2.1 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the lowest standard (standard 1 + 2 SD, repeatability, n = 21).

Expected values

Investigator	Therapeutic (µg/mL)	Therapeutic (µmol/L)
Penry and Newmark[3]	5-12	21.2-50.8
Scheuer and Pedley[4]	8-12	33.8-50.8
Troupin et al.[12]	8-12	33.8-50.8
Strandjord and Johannessen[13]	3-12	12.7-50.8
Simonsen et al.[14]	6-10	25.4-42.3
Larkin et al.[15]	4-10	16.9-42.3
Shorvon et al.[16]	4-8	16.9-33.8
MacKichan and Kutt[17]	4-12	16.9-50.8

Equivalent diagnostic technologies have shown that in most adults receiving carbamazepine as the sole antiepileptic agent, a peak therapeutic response is achieved with levels between 8-12 µg/mL (33.8-50.8 µmol/L). Lower concentrations may provide effective therapeutic response when other anticonvulsants are used in combination with carbamazepine.[16,18]

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.

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 a modified NCCLS EP5-T2 protocol (repeatability n = 63 and intermediate precision n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum/Plasma

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Control 1	3.58 (15.1)	0.14 (0.6)	3.8
Control 2	9.87 (41.8)	0.19 (0.8)	1.9
Control 3	15.5 (65.6)	0.2 (0.8)	1.5
HS 1	5.14 (21.7)	0.18 (0.8)	3.4
HS 2	10.9 (46.1)	0.2 (0.8)	2.0

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Control 1	3.58 (15.1)	0.23 (1.0)	6.4
Control 2	9.87 (41.8)	0.26 (1.1)	2.6
Control 3	15.5 (65.6)	0.3 (1.3)	2.1
HS 1	5.14 (21.7)	0.24 (1.0)	4.7
HS 2	10.9 (46.1)	0.3 (1.3)	2.7

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Method comparison

Serum/plasma

Carbamazepine values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and COBAS INTEGRA reagent on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 60
Passing/Bablok[19]	Linear regression
y = 0.972x + 0.030 µg/mL	y = 0.974x + 0.002 µg/mL
τ = 0.958	r = 0.998

The sample concentrations were between 0.520 and 19.4 µg/mL (2.20 and 82.1 µmol/L).

COBAS INTEGRA 800 analyzer	Sample size (n) = 56
Passing/Bablok[19]	Linear regression
y = 0.935x + 0.525 µg/mL	y = 0.925x + 0.590 µg/mL
τ = 0.942	r = 0.997

The sample concentrations were between 0.530 and 18.8 µg/mL (2.24 and 79.5 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Amitriptyline	100	18.6
Nortriptyline	50	17.2
Phenothiazine	200	8.6
Carbamazepine-10, 11-epoxide	250	7.4
Imipramine	200	5.6
Diazepam	250	4.8
Probenecid	500	2.0
Methosuximide	1000	1.0
Oxcarbazepine	15	-6.13
MHD	15	-4.20

Cross-reactivity is less than 1.0 % for the compounds listed below.

Amobarbital	Phenytoin
Chlorazepate	5-(p-Hydroxyphenyl)-phenylhydantoin
Chlordiazepoxide	p-Hydroxyphenobarbital
Ethosuximide	Primidone
Ethotoin	Promethazine
Glutethimide	Secobarbital
Mephenytoin	Valproic Acid
2-Phenyl-2-ethylmalonamide	

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
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

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.

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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.

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