PHNO2

cobas®

ONLINE TDM Phenobarbital

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490924190	04490924500	ONLINE TDM Phenobarbital (100 Tests)	System-ID 07 6915 0	**cobas c** 311, **cobas c** 501/502
05027446190	04490924500	ONLINE TDM Phenobarbital (200 Tests)	System-ID 07 6915 0	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	System-ID 07 6830 8 Codes 691-696	
04521536190	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	

English

System information

For **cobas c** 311/501 analyzers:
PHNO2: ACN 508

For **cobas c** 502 analyzer:
PHNO2: ACN 8508

Intended use

In vitro test for the quantitative determination of phenobarbital in serum and plasma on **cobas c** systems.

Summary

Phenobarbital measurements, performed with this assay in human serum and plasma, are used in monitoring phenobarbital levels to ensure appropriate therapy.

Phenobarbital belongs to the anti-epileptic drugs, also referred to as anticonvulsants or antiseizure medications. It is used as mono- and polytherapy in the treatment of neurological conditions such as seizures, except absence seizures, and as a sedative (for more detailed information on indications, refer to the drug information).[1,2,3]

In epileptic patients, the clinical end point (absence of seizures) can only be assessed prospectively, namely if the patient remains without seizures. Therefore, monitoring of anti-epileptic drugs is used to reduce the risk of drug toxicity on one hand, and on the other hand to indicate likely therapeutic concentrations.[4] For phenobarbital, monitoring is useful due to a high variability in phenobarbital clearance and variable relationships of dose to plasma concentrations.[2] Due to drug-drug-interactions, monitoring is especially recommended when co-administered with drugs that may enhance toxicity such as phenytoin and valproic acid.[3]

Furthermore, phenobarbital is a major metabolite of primidone, another antiseizure medication. It accumulates in the circulation in patients treated with primidone and contributes to its therapeutic effect.[1,2] In case, primidone is not taken as indicated, primidone concentrations may be increased in relation to the phenobarbital concentration.[5] Therefore, measuring both, phenobarbital and primidone, can be useful in chronic treatment to confirm adherence to medication.[1,2,6]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Phenobarbital antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of phenobarbital in the sample. A competitive reaction takes place between the drug conjugate and phenobarbital in the serum sample for binding to the phenobarbital antibody on the microparticles. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

Reagents - working solutions

R1 Phenobarbital conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.85; preservative; stabilizer

R2 Anti-phenobarbital antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino) propane sulfonic acid (MOPS) buffer, pH 7.4; stabilizer; preservative

R1 is in position B and R2 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.

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:	90 days

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- or K_3-EDTA, lithium or sodium heparin.

Stability:	7 days capped at 15-25 °C,
	7 days capped at 2-8 °C
	1 year capped at -20 °C (± 5 °C)[7]

Freeze only once.

Do not induce foaming of specimens.

Invert thawed specimens several times prior to testing.

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.

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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	2-Point end		
Reaction time /Assay points:	10 / 10-49		
Wavelength (sub/main)	800 /600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	93 µL	–	
R2	93 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	2-Point end		
Reaction time /Assay points:	10 / 16-60		
Wavelength (sub/main)	800 /600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	93 µL	–	
R2	93 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Calibration

Calibrators	S1-6 Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6-point calibration - after reagent lot change - every 6 weeks - 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 phenobarbital 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

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor:[7] µg/mL x 4.31 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at phenobarbital levels of approximately 15 and 40 µg/mL (65 and 172 µmol/L).

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

Hemolysis:[8] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8] No significant interference up to an L index of 600. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Triglycerides: No significant interference from triglycerides up to a concentration of 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 200 IU/mL.

Total protein: No significant interference from total protein up to a concentration of 14 g/dL.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[9]

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. 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 required in certain cases.

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

Limits and ranges

Measuring range

2.4-60 µg/mL (10.3-258.6 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.

Lower limits of measurement

Lower detection limit of the test

1.2 µg/mL (5.2 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 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 21).

Expected values

The therapeutic range of phenobarbital is correlated with seizure control as well as the absence of toxic effects, and is generally accepted to be between 10 and 30 µg/mL (43.1 and 129 µmol/L). Variation in metabolism and absorption of the drug may cause levels to rise above 40 µg/mL

(172 µmol/L) or fall below 15 µg/mL (64.7 µmol/L). The most frequent dose-related side effect is sedation, to which a tolerance usually develops. Phenobarbital serum levels above 40 µg/mL (172 µmol/L) are often associated with nystagmus, ataxia, and dysarthria.[10,11] At high doses, phenobarbital can even cause an increase in seizure frequency.

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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, intermediate precision n = 63). The following results were obtained on the **cobas c** 501 analyzer:

Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	9.8	42.2	0.5	2.1	5.0
Control 2	24.4	105	0.6	3	2.4
Control 3	45.1	194	0.8	3	1.8
HS 1	15.6	67.2	0.5	2.3	3.4
HS 2	37.8	163	1.0	4	2.7

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	9.8	42.2	0.5	2.3	5.4
Control 2	24.4	105	0.6	3	2.4
Control 3	45.1	194	0.9	4	2.0
HS 1	15.6	67.2	0.6	2.7	3.9
HS 2	37.8	163	1.2	5	3.0

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

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

Roche/Hitachi 917	Sample size (n) = 53
Passing/Bablok[12]	Linear regression
y = 0.998x - 0.206 µg/mL	y = 0.982x - 0.077 µg/mL
τ = 0.936	r = 0.996

The sample concentrations were between 2.91 and 57.7 µg/mL (12.5 and 249 µmol/L).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Functional sensitivity

2.4 µg/mL (10.3 µmol/L)

The functional sensitivity is calculated as the lowest concentration from clinical samples with a CV of ≤ 20 %.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Acetylsalicylic acid	1000	ND
Amitriptyline	9	ND
Amobarbital	1000	ND
Aprobarbital	1000	ND
Barbital	1000	ND
Butabarbital	1000	0.15
Butalbital	1000	0.67
Caffeine	1000	ND
Carbamazepine	1000	ND
Carbamazepine-10,11-epoxide	140	ND
Chlordiazepoxide	30	ND
Chlorpromazine	50	ND
Clonazepam	1.2	ND
5,5 Diallybarbituric acid	1000	ND
Diazepam	25	ND
Ethosuximide	1000	ND
Glutethimide	1000	ND
Hexobarbital	1000	ND
5-(p-Hydroxyphenyl)-5-phenylhydantoin	1000	ND
Imipramine	5	ND
Meperidine-HCl	100	ND
Mephenytoin	1000	ND
Mephobarbital	1000	0.18
Methsuximide	400	ND
Methyprylon	1200	ND
Nitrazepam	0.6	ND
Nordiazepam	100	ND
Pentobarbital-Na	1000	ND
Phensuximide	1000	ND
Phenylbutazone	2500	ND
2-Phenyl-2-ethylmalonamide (PEMA)	1000	ND
Phenytoin	1000	ND
P-Hydroxyphenobarbital	200	ND
Primidone	120	ND
Promethazine	0.23	ND
Secobarbital	1000	0.15
Theophylline	200	ND
Thiopental-Na	1000	ND
Valproic acid	1000	ND

Cross-reactivity was designated as “Not Detectable” (ND) if the obtained value was less than the sensitivity of the assay.

Tests were performed on 18 drugs. No significant interference with the assay was found.

Acetaminophen	Heparin
Acetyl cysteine	Ibuprofen
Acetylsalycilic acid	Intralipid
Ampicillin-Na	Levodopa

Ascorbic acid	Methyldopa + 1.5 H_2O
Ca-Dobesilate	Metronidazole
Cefoxitin	Phenylbutazone
Cyclosporine	Rifampicin
Doxycycline (Tetracycline)	Theophylline

References

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2 Patsalos PN, Spencer EP, Berry DJ. Therapeutic Drug Monitoring of Antiepileptic Drugs in Epilepsy: A 2018 Update. Ther Drug Monit 2018 Oct;40(5):526-548.

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7 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;866.

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

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10 Kutt H, Penry JK. Usefulness of blood levels of anti-epileptic drugs. Arch Neurol 1974;31:283-288.

11 Morselli PL. Antiepileptic Drugs in Drug Disposition During Development. Morselli PL, ed. New York, NY: Spectrum 1971;311-360.

12 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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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 for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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

CE 0123

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