Phenobarbital

PHNO3

cobas®

• Indicates **cobas c** systems on which reagents can be used

Order information			Roche/Hitachi **cobas c** systems
			cobas c 501
CEDIA Phenobarbital			
125 Tests	Cat. No. **04874617** 190	System-ID 07 6951 7	•
CEDIA Core TDM Multi-Cal	Cat. No. **11815253** 216		
Low	2 x 7.5 mL	Code 662	
High	2 x 5 mL	Code 663	
TDM Control Set	Cat. No. **04521536** 190		
Level I	2 x 5 mL	Code 310	
Level II	2 x 5 mL	Code 311	
Level III	2 x 5 mL	Code 312	
Opening tool	See Transfer instruction sheet included in kit		

English

System information
PHNO3: ACN 277

Intended use
The CEDIA Phenobarbital assay is for the quantitative determination of phenobarbital in human serum and plasma on Roche/Hitachi **cobas c** systems. Measurements are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of drug to ensure proper therapy.

Summary
Phenobarbital has been widely prescribed for the treatment of epilepsy, particularly for controlling focal motor or sensory and grand mal seizures,[1] since the discovery of phenobarbital by Hauptmann in 1912.[2] After oral doses of 2 to 3 mg/kg, phenobarbital is almost completely absorbed with peak levels achieved by 12 to 18 hours.[3] Phenobarbital in circulation is approximately 40 to 50 percent bound to plasma protein with a relatively low association constant.[4] The major metabolic pathway of phenobarbital is hydroxylation of the phenyl ring to parahydroxy phenobarbital, an agent devoid of hypnotic activity, which is then excreted in the urine in equal amounts of the free form and the conjugated form with glucuronic acid.[4] The antiepileptic properties of phenobarbital have not yet been explained by a specific mechanism of action. Principally, phenobarbital diminishes the excitability of neurons and reduces excitatory postsynaptic potentials.[3] Monitoring phenobarbital concentrations in serum is essential during therapy in order to provide physicians with an indicator for adjusting dosage.[5] The need for monitoring phenobarbital concentrations is also due to the narrow therapeutic index and wide variability in individual rate of drug absorption, metabolism and clearance.[6] Toxicity of phenobarbital often associated with therapy may or may not be dose-related. Most of the dose-related toxic effects are neurologic, including sedation, nystagmus, ataxia and coma.[3,7] Toxic effects that may not be dose-related include sedation, paradoxical excitement, blood dyscrasia (including the coagulation defects of neonates of mothers given the drug during pregnancy), nonspecific hepatic changes, rash (including severe exfoliative forms), osteomalacia and the shoulder-hand syndrome.[8,9] Recently, the study by Farwell, et al.[10] also demonstrated that lower measured intelligence in children was observed with those assigned to long term phenobarbital therapy. In combination with other clinical information, monitoring serum or plasma phenobarbital levels provides physicians and patients with an essential tool to aid in adjusting dosage, and achieving optimal therapeutic effect, while avoiding both subtherapeutic and harmful toxic drug levels.

Test principle[11]
The CEDIA Phenobarbital 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: EA (Enzyme Acceptor) and ED (Enzyme Donor). 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, the displacement format is used to provide improved sensitivity and precision through reduced background noise and concomitant increase of signal-to-noise ratio. In the assay, phenobarbital in the sample displaces a fraction of the antibody ED-phenobarbital conjugate complex. Subsequently, EA reagent is added and the reactants are incubated to allow complementation with free ED-phenobarbital conjugate. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of phenobarbital present in the sample.

Reagents - working solutions

R1 ED working solution
enzyme donor (microbial)-phenobarbital: 38 µg/L; chlorophenol red-β-D-galactopyranoside: 1.64 g/L; anti-phenobarbital antibody (mouse monoclonal): 16.1 mg/L; 3-(N-morpholino)propanesulfonic acid (MOPS) buffer; buffer salts; preservative

R2 EA working solution
enzyme acceptor (microbial): 0.171 g/L; 3-(N-morpholino) propanesulfonic acid (MOPS) buffer; buffer salts; goat anti-mouse antibodies: 18 mg; stabilizer; preservative

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
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 according to the European Directive 88/379 EEC:
☒ Xn - Harmful (sodium azide) R 22; S 7, S 13, S 24
Harmful if swallowed. Keep container tightly closed. Keep away from food, drink and animal feeding stuffs. Avoid contact with the skin.
Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336.
Safety data sheet available for professional user on request.
Disposal of all waste material should be in accordance with local guidelines.

Reagent handling
For reagent handling instructions, refer to the **cobas c** pack Transfer instruction sheet contained in the reagent kit.

NOTE 1: The components supplied in the kit are intended for use as an integral unit. Do not mix components from different lots.
NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R1 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: The R1 and R2 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
Do not freeze.
On-board in use and refrigerated on the analyzer: 60 days
To ensure reconstituted EA reagent stability, protect from prolonged continuous exposure to bright light.

Phenobarbital

PHNO3

cobas®

Specimen collection and preparation
For specimen collection and preparation, only use suitable tubes or collection vessels. Some gel separation tubes may not be suitable for use with therapeutic drug monitoring assays. Avoid repeated freezing and thawing. Do not induce foaming of specimens. Centrifuge samples containing precipitate before performing the assay.
When processing samples in primary tubes, follow the instructions of the tube manufacturer.
Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sampling tubes.
Plasma: Sodium or lithium heparin or sodium EDTA plasma.

Stability:[12] 2 days capped at 2-8°C
3-12 months capped at -20°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 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 this application in the Utility menu, Application screen, Range tab.

cobas c 501 **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	2.1 µL	–	–
Decreased	2.1 µL	–	–
Increased	2.1 µL	–	–
Instrument factor	Set instrument factor a = 0.97 on the Calibration/Status/Instrument Factor display		

Calibration

Calibrator	CEDIA Core TDM Multi-Cal calibrator
Calibration mode	Linear
Calibration frequency	2 point calibration - after **cobas c** pack change - after reagent lot change - and 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 bovine serum albumin.

Quality Control
For quality control, use control materials as listed in the "Order information" section.
Other suitable control material can be used in addition.
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 limits.

Calculation
Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.
Conversion factor: µg/mL x 4.31 = µmol/L[13]

Limitations - interference
Criterion: recovery within ±1.2 µg/mL (±5.2 µmol/L) of initial value at concentrations <12 µg/mL (<51.7 µmol/L) or ±10% of initial value at concentrations >12 µg/mL (>51.7 µmol/L).

Serum/Plasma
Icterus: No significant interference up to an I index of 60 (approximate bilirubin concentration: 60 mg/dL or 1026 µmol/L).

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

Lipemia (Intralipid): 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 12.8 g/dL.

Mephobarbital (>100%) and amobarbital (>20%) show significant cross-reactivity.
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 phenobarbital results that do not fit the clinical profile. If this occurs, contact Customer Technical Support.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
1.2-80.0 µg/mL (5.2-344.8 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.

Analytical sensitivity[8]
1.2 µg/mL (5.2 µmol/L)
The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (standard 1 + 2 SD, within-run precision, n = 21).

Expected values
In most patients, a therapeutic response is achieved with phenobarbital concentrations in the 15-40 µg/mL (65-172 µmol/L)[14] range. Some patients may require serum or plasma levels outside this range to obtain effective seizure control due to patient to patient differences in metabolic activity. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Specific performance data[15]
Representative performance data on a Roche/Hitachi analyzer are given below. Results obtained in individual laboratories may differ.

Phenobarbital

PHNO3

cobas®

Precision

Reproducibility was determined using controls and human samples in a modified NCCLS EP5-T2 protocol (within run n = 63, total n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum/Plasma

Within run	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	9.2	39.7	0.22	0.95	2.4
Control 2	23.2	99.9	0.43	1.85	1.8
Control 3	45.4	195.7	0.65	2.80	1.4
HS 1	18.3	78.9	0.46	1.98	2.5
HS 2	32.4	139.6	0.57	2.46	1.8

Total	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	9.2	39.7	0.35	1.51	3.8
Control 2	23.2	99.9	0.67	2.89	2.9
Control 3	45.4	195.7	0.92	3.97	2.0
HS 1	18.3	78.9	0.66	2.84	3.6
HS 2	32.4	139.6	0.87	3.75	2.7

Method comparison

Serum/plasma

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

Roche/Hitachi 917 analyzer	Sample size (n) = 70
Passing/Bablok[16]	Linear regression
y = 0.988 x - 0.019 µg/mL	y = 0.985 x - 0.008 µg/mL
τ = 0.965	r = 0.999

The sample concentrations were between 1.9 and 42.9 µg/mL (8.2 and 184.9 µmol/L).
τ = Kendall's tau.

COBAS INTEGRA 800 analyzer	Sample size (n) = 70
Passing/Bablok[16]	Linear regression
y = 1.096 x - 2.045 µg/mL	y = 1.089 x - 1.767 µg/mL
τ = 0.963	r = 0.997

The sample concentrations were between 1.8 and 40.5 µg/mL (7.8 and 174.6 µmol/L).
τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity:

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Secobarbital	2000	2.2
Aprobarbital	1000	≤5.3
Butabarbital	1000	≤2.3
Barbital	2000	≤1.6
Phenytoin	400	≤0.9
Valproic Acid	2000	≤0.7
Pentobarbital	1000	≤0.6
Primidone	1000	≤0.5
Amitryptyline	1000	<0.12
Carbamazepine	1000	<0.12
Carbamazepine-10,11 epoxide	1000	<0.12
Chlorazepate	2000	<0.12
Chlorpromazine	1000	<0.12
Diazepam	1000	<0.12
1,3-Dimethylbarbituric acid	1000	<0.12
Ethosuximide	1000	<0.12
Ethotoin	1000	<0.12
Glutethimide	1000	<0.12
ρ-Hydroxyphenobarbital	2000	<0.12
5-(ρ-Hydroxyphenyl)-5-phenylhydantoin	1000	<0.12
Imipramine	2000	<0.12
Mephenytoin	1000	<0.12
Methsuximide	1000	<0.12
2-Phenyl-2-ethylmalonamide	1000	<0.12
Promethazine	1000	<0.12
Sulthiame	1000	<0.12

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
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

References

1. Buchthal F, Lennox-Buchthal MA. Relation of serum concentration to control of seizures. In: Woodbury DM, Penry JK, Schmidt KP, eds. Antiepileptic Drugs New York, NY: Raven Press; 1972: 335-343.
2. Hauptmann A. Luminal bei Epilepsy. Munch Med Wschr 1912;59:1907-1909.
3. Penry JK, Newmark ME. The use of anti-epileptic drugs. Ann Int Med 1979;90:207-218.
4. Glazko AJ. Antiepileptic drugs: biotransformation, metabolism, and serum half-life. Epilepsia 1975;16:367-391.
5. Kutt H, Penry K. Usefulness of blood levels of antiepileptic drugs. Arch Neurol 1974;3:283-288.
6. Waddell WJ, Butler TC. The distribution and excretion of phenobarbital. J Clin Invest 1957;36:1217-1226.
7. Buchthal F, Svensmark O. Aspects of the pharmacology of phenytoin (Dilantin) and phenobarbital relevent to their dosage in the treatment of epilepsy. Epilepsia 1960;1:373-384.
8. Eadie MJ, Tyrer JH. Anticonvulsant therapy: pharmacological basis and practice. London, England: Churchill Livingstone; 1974.

9. Dent CE, Richens A, Rowe DJF, et al. Osteomalacia with long-term anticonvulsant therapy in epilepsy. Brit Med J 1970;4:69-72.
10. Farwell JR, Lee YJ, Hirtz DG, et al. Phenobarbital for febrile seizures-effects on intelligence and on seizure recurrence. N Engl J Med 1990;322:363-369.
11. Henderson DR, Friedman SB, Harris JD, et al. CEDIA™, a new homogeneous immunoassay system. Clin Chem 1986;32(9): 1637-1641.
12. Clinical Laboratory Handbook for Patient Preparation and Specimen Handling. Therapeutic Drug Monitoring and Toxicology Fascicle IV 1985.
13. Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, Pa: WB Saunders Co; 1995:866.
14. Buchthal F, Svensmark O. Serum concentrations of diphenylhydantoin (phenytoin) and phenobarbital and their relation to therapeutic and toxic effects. Psychiatr Neurol Neurochir 1971;74:117-135.
15. Data on file at Roche Diagnostics.
16. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

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, D-68298 Mannheim

Assembled and distributed by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

Roche