PHNY2

cobas®

Phenytoin

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490932 190	ONLINE TDM Phenytoin (100 Tests)	System-ID 07 6925 8	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
05108411 190	ONLINE TDM Phenytoin (200 Tests)	System-ID 07 6925 8	
03375790 190	Preciset TDM I calibrators 1) CAL A-F (1 x 5 mL) 2) Diluent (1 x 10 mL)	Codes 691-696	
04521536 190	TDM Control Set 1) Level I (2 x 5 mL) 2) Level II (2 x 5 mL) 3) Level III (2 x 5 mL)	Code 310 Code 311 Code 312	

English

System information

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

PHNY2: ACN 772

For **cobas c** 502 analyzer:

PHNY2: ACN 8772

Intended use

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

Summary

Phenytoin (diphenylhydantoin) has been used extensively for seizure control in patients having both grand mal epilepsy (major motor), cortical focal seizures, and temporal lobe epilepsy.[1] Serum level monitoring of the drug is essential in order to achieve maximal seizure control while maintaining minimal blood levels.[1,2,3,4,5,6,7] Because of individual variation in absorption and metabolism, optimum levels may vary.

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Phenytoin 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 phenytoin in the sample. A competitive reaction takes place between the drug conjugate and phenytoin in the serum sample for binding to the phenytoin 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 Phenytoin conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.3; stabilizer; preservative

R2 Anti-phenytoin 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.
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:	12 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: Sodium or lithium heparin, K_2- or K_3-EDTA plasma.

Stability:[8]	4 days capped at 2-8 °C or 20-25 °C
	1-2 months 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.[9] Due to the observed cross-reactivity of this assay to fosphenytoin, it is recommended that samples for serum phenytoin measurements be collected at least 2 hours after an intravenous dose of fosphenytoin and at least 4 hours after an intramuscular dose.[10]

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)

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R1	93 µL	–	
R2	93 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.7 µL	–	–
Decreased	1.7 µL	–	–
Increased	1.7 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	1.7 µL	–	–
Decreased	1.7 µL	–	–
Increased	1.7 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 phenytoin 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:[11] µg/mL x 3.96 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at phenytoin levels of approximately 5 µg/mL (19.8 µmol/L) and 20 µg/mL (79.2 µmol/L).

Serum/Plasma

Icterus:[12] No significant interference up to an I index of 50 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 50 mg/dL or 855 µmol/L).

Hemolysis:[12] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid):[12] No significant interference up to an L index of 800. 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 100 IU/mL.

Total protein: No interference from total protein up to 14 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 low results.

In rare instances (< 1 %), samples may contain unidentified component(s) which cause non-specific agglutination in this assay. These samples could give erroneously low phenytoin values. If a value is obtained which is inconsistent with the patient's clinical presentation, the result should be confirmed by an alternate method and the local Roche Diagnostics representative or Roche Customer Technical Support should be contacted.

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 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

0.8-40 µg/mL (3.2-158.4 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

0.8 µg/mL (3.2 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 phenytoin is correlated with seizure control as well as the absence of toxic effects, and is generally accepted to be between 10 and 20 µg/mL (39.6 and 79.2 µmol/L).[14,15,16] Because of individual variation in absorption and metabolism of the drug, optimum levels may vary and reach higher than 20 µg/mL (79.2 µmol/L) or they may fall below 10 µg/mL (39.6 µmol/L). Toxic signs are seldom seen below 15 µg/mL (59.4 µmol/L), while nystagmus often appears when serum levels rise above 20 µg/mL (79.2 µmol/L). Ataxia is observed most often when serum levels reach 25 to 30 µg/mL (99 to 119 µmol/L) and somnolence and dysarthria above 40 µg/mL (158 µmol/L). At high doses, phenytoin can even cause an increase in the frequency of seizures.[17]

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 a **cobas c** 501 analyzer.

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Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.78	26.8	0.23	0.9	3.4
Control 2	13.0	51.5	0.3	1.2	2.2
Control 3	22.9	90.7	0.6	2.3	2.5
HS 1	3.29	13.0	0.15	0.6	4.4
HS 2	20.0	79.2	0.5	1.9	2.4

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.78	26.8	0.26	1.0	3.8
Control 2	13.0	51.5	0.4	1.7	3.4
Control 3	22.9	90.7	0.8	3.3	3.6
HS 1	3.29	13.0	0.19	0.8	5.6
HS 2	20.0	79.2	0.9	3.7	4.7

Method comparison

Serum/plasma

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

Roche/Hitachi 917 analyzer	Sample size (n) = 78
Passing/Bablok[18]	Linear regression
y = 0.946x + 0.143 µg/mL	y = 0.958x – 0.051 µg/mL
τ = 0.953	r = 0.993

The sample concentrations were between 2.81 and 39.9 µg/mL (11.1 and 158 µmol/L).

COBAS INTEGRA 800 analyzer	Sample size (n) = 79
Passing/Bablok[18]	Linear regression
y = 1.016x + 0.066 µg/mL	y = 1.024x + 0.127 µg/mL
τ = 0.943	r = 0.993

The sample concentrations were between 2.65 and 39.6 µg/mL (10.5 and 157 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (µg/mL)**	**% Cross-reactivity**
Fosphenytoin	40	28.7
m-HPPH	500	5.2
p-HPPH	500	1.7
5-(*p*-methylphenyl)-phenylhydantoin	500	1.5
Amitryptyline	3000	ND
Amobarbital	1000	ND
Carbamazepine	500	ND
Carbamazepine 10,11 epoxide	1000	ND
Chlordiazepoxide	2000	ND
Chlorpromazine	2500	ND
Ethosuximide	1000	ND
Ethotoin	1000	ND
Glutethimide	500	ND
Hydantoin	2000	ND
10-Hydroxycarbamazepine (MHD)	150	ND
p-Hydroxyphenobarbital	1000	ND
Imipramine	4000	ND
Mephenytoin	3000	ND
Mephobarbital	1000	ND
Methsuximide	5000	ND
Oxaprozin	500	ND
Oxcarbamazepine (OXC)	150	ND
PEMA	1000	ND
Pentobarbital	1000	ND
Phenobarbital	2000	ND
Phensuximide	2000	ND
Primidone	1000	ND
Promethazine	1500	ND
Secobarbital	1000	ND
Valproic Acid	7000	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
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

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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.

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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