

## Phenytoin

## Order information

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20737879 322	Phenytoin (200 tests)	System-ID 07 3787 9 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	System-ID 07 6900 2 System-ID 07 6901 0 System-ID 07 6902 9
20720720 322	COBAS FP Sample Dilution Reagent II (1 × 200 mL)	System-ID 07 2072 0

## English

## System information

Test PHNYM, test ID 0-887.

## Intended use

In vitro diagnostic test for the quantitative determination of phenytoin in serum or heparinized plasma on COBAS INTEGRA 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.<sup>1</sup> Serum level monitoring of the drug is essential in order to achieve maximal seizure control while maintaining minimal blood levels.<sup>1,2,3,4,5,6,7</sup> Because of individual variation in absorption and metabolism, optimum levels may vary.

## Test principle

Fluorescence polarization

COBAS INTEGRA therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.<sup>8</sup> Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum for the purpose of therapeutic drug monitoring.

Surface active agents are used to ensure dissociation of the drug from serum proteins and to prevent nonspecific binding of the tracer.

## Reagents - working solutions

- R1** Antibody reagent (liquid)  
Anti-phenytoin monoclonal antibody (mouse) in buffer, pH 6.5, with stabilizer and preservative.
- SR** Tracer reagent (liquid)  
Fluorescein-labeled phenytoin derivative in buffer, pH 7.5, with stabilizer, surfactant, and preservative.

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

## Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

## Reagent handling

Ready for use

## Storage and stability

Shelf life at 2-8 °C

See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C

12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C

26 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

## 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:

Unhemolyzed serum

Unhemolyzed heparinized plasma

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.

Usual sampling time varies dependent upon route of delivery and desired measurement of peak or trough values.<sup>9</sup> Specimens should be tested within 8 hours of collection if kept at room temperature. If specimens must be stored for later testing, they may be kept at 2-8 °C for up to 48 hours or at -20 °C or below for longer periods. Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

COBAS FP Sample Dilution Reagent (SDR II), Cat. No. 20720720322

The SDR II is placed as special diluent in its predefined rack position and is stable for 7 days on-board COBAS INTEGRA 400 plus/800 analyzers.

## 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.

## Application for serum and plasma

## COBAS INTEGRA 400 plus test definition

Measuring mode	FP
Reaction mode	R1-SDR/S-SR
Wavelength	excitation 485 nm emission 515 nm
Reading cycle blank/test	29/45

## Phenytoin

Unit	µg/mL	
<b>Pipetting parameters</b>		
		Diluent (H <sub>2</sub> O)
R1	115 µL	10 µL
Sample	2 µL	5 µL
Special diluent SDR II	18 µL	
SR	15 µL	10 µL
Total volume	175 µL	

**COBAS INTEGRA 800 test definition**

Measuring mode	FP
Reaction mode	R1-SDR/S-SR
Wavelength	excitation 485 nm
	emission 515 nm
Reading cycle blank/test	40/60
Unit	µg/mL

**Pipetting parameters**

		Diluent (H <sub>2</sub> O)
R1	135 µL	10 µL
Sample	2 µL	5 µL
Special diluent SDR II	18 µL	
SR	15 µL	10 µL
Total volume	195 µL	

**Calibration**

Calibrators	Preciset TDM I
	Calibrators A-F
Calibration mode	Exponential 5
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 2.5 µg/mL (≥ 9.9 µmol/L)
Calibration interval	Each lot, every 26 weeks, and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of phenytoin in normal human serum and are traceable to USP reference standards.

**Note**

Calibrators should be assayed within 2 hours after placing on-board the instrument.

**Quality control**

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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.

**Note**

Controls should be assayed within 2 hours after placing on-board the instrument.

**Calculation**

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/mL × 3.96 = µmol/L

**Limitations - interference**

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors). Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate dilution of the original sample with the Preciset TDM I Diluent (0 µg/mL). Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

**Serum/plasma**

Criterion: Recovery within ± 10 % of initial value at a phenytoin concentration of 3.7 µg/mL (14.6 µmol/L) and 15 µg/mL (59.4 µmol/L).

Icterus:<sup>10</sup> No significant interference up to a bilirubin concentration of 498 µmol/L or 29.1 mg/dL.

Hemolysis:<sup>10</sup> No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:<sup>10</sup> No significant interference up to a triglycerides concentration of 2763 mg/dL.

Total protein: No significant interference up to a total protein concentration of 4-12 g/dL.

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

**Limits and ranges****Measuring range**

COBAS INTEGRA 400 plus analyzer:

0.42-40 µg/mL (1.7-158 µmol/L)

COBAS INTEGRA 800 analyzer:

0.61-40 µg/mL (2.4-158 µmol/L)

**Lower limits of measurement**

Lower detection limit of the test:

COBAS INTEGRA 400 plus analyzer:

0.42 µg/mL (1.7 µmol/L)

COBAS INTEGRA 800 analyzer:

0.61 µg/mL (2.4 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

**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 10-20 µg/mL (39.6-79.2 µmol/L).<sup>11,12,13</sup> 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

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25-30 µg/mL (99-119 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.<sup>14</sup>

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 COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

## Precision

Precision was determined using controls in accordance with the NCCLS EP5-T2<sup>15</sup> requirements with repeatability (n = 80) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on a COBAS INTEGRA 700 analyzer.

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	5.6 (22.2)	0.13 (0.51)	2.3
Level 2	16.2 (64.2)	0.35 (1.39)	2.1
Level 3	27.8 (110)	0.62 (2.46)	2.2

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	5.6 (22.2)	0.16 (0.63)	2.8
Level 2	16.2 (64.2)	0.39 (1.54)	2.4
Level 3	27.8 (110)	0.62 (2.46)	2.2

## Method comparison

Phenytoin values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Phenytoin reagent (y) were compared with those determined using a commercially available FPIA method (x).

	FPIA
Number of samples	209
Range of values	min. 1.1 µg/mL max. 34.4 µg/mL
Slope	1.068
Intercept	-1.596 µg/mL
Correlation coefficient	0.992

## Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with phenytoin at 19 µg/mL (75.2 µmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.<sup>16</sup> The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested µg/mL	Cross-reactivity %
Fosphenytoin (Cerebyx)	6.25	224
5-p-(Hydroxyphenyl)- 5-phenylhydantoin (HPPH)	220	2.5
Mephencytoin	400	ND
Phenobarbital	400	0.1
2-Phenyl-2-ethyl- malonamide (PEMA)	100	ND

ND = Not Detectable

In a similar study, the following structurally related or potentially coadministered compounds were tested on the COBAS FARA II analyzer using normal human serum spiked with phenytoin at 20 µg/mL (79.2 µmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
Amobarbital	10	ND
Butobarbital	250	ND
Carbamazepine	140	ND
Carbamazepine-10,11-epoxide	140	ND
Diphenhydramine	400	ND
Ethosuximide	1000	ND
p-Hydroxyphenobarbital	300	ND
Pentobarbital	50	ND
Phenylbutazone	450	ND
Primidone	120	ND
d-Propoxyphene	400	0.1

ND = Not Detectable

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

## References

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- 16 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

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 (for USA: see <https://usdiagnostics.roche.com> for definition of symbols used):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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