

| REF          | CONTENT  | Analyzer(s) on which <b>cobas c</b> pack(s) can be used            |
|--------------|--|--|
| 20737860 322 | Phenobarbital (200 tests)  | System-ID 07 3786 0<br>COBAS INTEGRA 400 plus<br>COBAS INTEGRA 800 |
| 03375790 190 | Preciset TDM I<br>Calibrators A-F (6 × 1 × 5 mL)<br>Diluent (1 × 10 mL)              | System-ID 07 6830 8  |
| 04521536 190 | TDM Control Set<br>Level I (2 × 5 mL)<br>Level II (2 × 5 mL)<br>Level III (2 × 5 mL) | System-ID 07 6900 2<br>System-ID 07 6901 0<br>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 PHNOM, test ID 0-286.

## Intended use

In vitro diagnostic test for the quantitative determination of phenobarbital in serum or heparinized plasma on COBAS INTEGRA systems.

## Summary

Phenobarbital is one of the most commonly used drugs for the treatment of grand mal, psychomotor epilepsy, and other forms of focal epilepsy. Monitoring of the serum level of the drug is essential in order to achieve maximal seizure control while maintaining minimal blood levels to avoid negative side effects.<sup>1,2,3,4,5,6,7,8,9</sup> As with other anti-convulsant drugs, it is imperative that each patient's dosage be individualized.<sup>10</sup>

## 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>11</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  
Anti-phenobarbital monoclonal antibody (mouse) in buffer, pH 7.5, with stabilizer and preservative.
- SR** Tracer reagent  
Fluorescein-labeled phenobarbital derivative in buffer, pH 6.5, with stabilizer 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.

## 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.

Specimens should be tested within 8 hours of collection if kept at room temperature. If specimens must be stored for later testing, they should be kept in a refrigerator 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. 20720720 322

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<br>emission 515 nm |
| Reading cycle blank/test | 29/45                                |
| Unit                     | µg/mL                                |

## Pipetting parameters

|    |        |                            |
|----|--------|----------------------------|
|    |        | Diluent (H <sub>2</sub> O) |
| R1 | 140 µL | 10 µL                      |

|                        |        |       |
|------------------------|--------|-------|
| Sample                 | 2 µL   | 5 µL  |
| Special diluent SDR II | 23 µL  |       |
| SR                     | 10 µL  | 10 µL |
| Total volume           | 200 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                     | 140 µL | 10 µL                      |
| Sample                 | 2 µL   | 5 µL                       |
| Special diluent SDR II | 23 µL  |                            |
| SR                     | 10 µL  | 10 µL                      |
| Total volume           | 200 µL |                            |

**Calibration**

|                       |  |
|-----------------------|--|
| Calibrators           | Preciset TDM I   |
|                       | Calibrators A-F  |
| Calibration mode      | Logit/log 5  |
| Calibration replicate | Duplicate recommended  |
| Deviation low/high    | < 10 % at ≥ 5 µg/mL (≥ 22 µmol/L)  |
| Calibration interval  | Each lot, every 20 weeks, and as required following quality control procedures |

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 phenobarbital 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 × 4.31 = 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 phenobarbital concentration of 8.7 µg/mL (37.5 µmol/L) and 32 µg/mL (138 µmol/L).

Icterus:<sup>12</sup> No significant interference up to a bilirubin concentration of 657 µmol/L or 38.4 mg/dL.

Hemolysis:<sup>12</sup> No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:<sup>12</sup> No significant interference up to a triglycerides concentration of 3193 mg/dL.

Total protein: No significant interference up to a total protein concentration of 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:

1.0-60 µg/mL (4.3-259 µmol/L)

COBAS INTEGRA 800 analyzer:

1.0-60 µg/mL (4.3-259 µmol/L)

**Lower limits of measurement**

Lower detection limit of the test:

1.0 µg/mL (4.3 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 phenobarbital is correlated with seizure control as well as the absence of toxic effects, and is generally accepted to be between 10-30 µg/mL (43.1-129 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.<sup>13,14</sup> At high doses, phenobarbital can even cause an increase in seizure frequency.

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<br>µg/mL (µmol/L) | SD<br>µg/mL (µmol/L) | CV<br>% |
|---------------|------------------------|----------------------|---------|
| Level 1       | 12.0 (51.7)            | 0.25 (1.08)          | 2.1     |
| Level 2       | 23.4 (101)             | 0.51 (2.20)          | 2.2     |
| Level 3       | 52.0 (224)             | 1.52 (6.55)          | 2.9     |

| Intermediate precision | Mean<br>µg/mL (µmol/L) | SD<br>µg/mL (µmol/L) | CV<br>% |
|------------------------|------------------------|----------------------|---------|
| Level 1                | 12.0 (51.7)            | 0.26 (1.12)          | 2.2     |
| Level 2                | 23.4 (101)             | 0.62 (2.67)          | 2.7     |
| Level 3                | 52.0 (224)             | 2.04 (8.79)          | 3.9     |

**Method comparison**

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

|                         | FPIA                              |
|-------------------------|-----------------------------------|
| Number of samples       | 206                               |
| Range of values         | min. 0.8 µg/mL<br>max. 60.0 µg/mL |
| Slope                   | 1.036                             |
| Intercept               | -1.236 µg/mL                      |
| Correlation coefficient | 0.995                             |

**Analytical specificity**

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with phenobarbital at 36 µg/mL (155 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<br>µg/mL | Cross-reactivity<br>% |
|----------------------------|-----------------------|-----------------------|
| Amobarbital                | 1000                  | 0.1                   |
| Aprobarbital               | 1000                  | 0.0                   |
| Butabarbital               | 1000                  | 0.4                   |
| 5,5 Diallylbarbituric acid | 1000                  | ND                    |
| Mephobarbital              | 1000                  | 0.3                   |
| Secobarbital               | 1000                  | 0.1                   |

ND = Not Detectable

In a similar study, the following structurally related or potentially co-administered compounds were tested on the COBAS FARA II analyzer using normal human serum spiked with phenobarbital at 37.5 µg/mL (162 µmol/L).

| Drug                 | Level tested<br>µg/mL | Cross-reactivity<br>% |
|----------------------|-----------------------|-----------------------|
| Acetylsalicylic acid | 1000                  | ND                    |
| Amitriptyline        | 9                     | ND                    |

| Drug                                      | Level tested<br>µg/mL | Cross-reactivity<br>% |
|---|-----------------------|-----------------------|
| Barbital                                  | 973                   | ND                    |
| Butalbital                                | 1000                  | ND                    |
| Caffeine                                  | 1000                  | ND                    |
| Carbamazepine                             | 1000                  | ND                    |
| Carbamazepine-10,11-epoxide               | 140                   | ND                    |
| Chlordiazepoxide                          | 30                    | ND                    |
| Chlorpromazine                            | 50                    | ND                    |
| Clonazepam                                | 1.2                   | ND                    |
| Diazepam                                  | 25                    | ND                    |
| Ethosuximide                              | 1000                  | ND                    |
| Glutethimide                              | 1000                  | ND                    |
| Hexobarbital                              | 1000                  | ND                    |
| 5-(p-Hydroxyphenyl)-<br>5-phenylhydantoin | 1000                  | ND                    |
| Imipramine                                | 5                     | ND                    |
| Meperidine                                | 100                   | ND                    |
| Mephenytoin                               | 1000                  | ND                    |
| Methsuximide                              | 400                   | 0.1                   |
| Methypylon                                | 1200                  | ND                    |
| Nitrazepam                                | 0.6                   | ND                    |
| Nordiazepam                               | 100                   | ND                    |
| Pentobarbital                             | 1000                  | ND                    |
| Phensuximide                              | 1000                  | ND                    |
| Phenylbutazone                            | 2500                  | ND                    |
| 2-Phenyl-2-ethylmalonamide<br>(PEMA)      | 1000                  | ND                    |
| Phenytoin                                 | 1000                  | ND                    |
| P-Hydroxyphenobarbital                    | 200                   | ND                    |
| Primidone                                 | 120                   | 0.3                   |
| Promethazine                              | 0.23                  | ND                    |
| Theophylline                              | 200                   | ND                    |
| Thiopental                                | 1000                  | ND                    |
| Valproic acid                             | 1000                  | 0.1                   |

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

**References**



- Johannessen SI. Antiepileptic drugs: pharmacokinetic and clinical aspects. *Thera Drug Monit* 1981;3(1):17-37.
- Koch-Weser J. Serum drug concentrations in clinical perspective. *Ther Drug Monit* 1981;3(1):3-16.
- Buchthal F, Lennox-Buchthal MA. In: *Antiepileptic Drugs*. Woodbury DM, Penry JK, Schmidt RP, eds. New York, NY: Raven Press 1972;193-209.
- Buchthal F, Svensmark O. Serum concentration of diphenylhydantoin (phenytoin) and phenobarbital and their relation to therapeutic and toxic effects. *Psychiatr Neurol Neurochir* 1971;74:117-136.
- Booker HE, Hosokawa K, Burdette RD, et al. A clinical study of serum primidone levels. *Epilepsia* 1970;11:395-402.
- Lund L. Anti-convulsant effect of diphenylhydantoin relative to plasma levels. *Arch Neurol* 1974;31:289-294.

- 7 Sherwin AD, Robb JP, Lechter M. Improved control of epilepsy by monitoring plasma ethosuximide. Arch Neurol 1973;28:178-181.
- 8 Penry JK, Smith LD, White BG. Clinical Value and Methods. DHEW Publication No 73-396 (NIH) USGPO, Washington, DC 1972.
- 9 Troupin A, Ojemann LM, Halpern L, et al. Carbamazepine - a double blind comparison with phenytoin. Neurology 1977;27:511-519.
- 10 Pippenger CE. Effective Seizure Control Requires Drug Monitoring. Battaglia BJ, ed. Clin Chem. New Special Section. Washington, DC: American Association of Clinical Chemistry 1980:1s and 10s.
- 11 Dandliker WB, Feigen GA. Quantification of the antigen-antibody reaction by the polarization of fluorescence. Biochem Biophys Res Comm 1961;5:299-304.
- 12 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 13 Kutt H, Penry JK. Usefulness of blood levels of anti-epileptic drugs. Arch Neurol 1974;31:283-288.
- 14 Morselli PL. Antiepileptic Drugs in Drug Disposition During Development. Morselli PL, ed. New York, NY: Spectrum 1971;311-360.
- 15 National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.
- 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.

|   |                                       |
|---|---------------------------------------|
|  | Contents of kit                       |
|  | Volume after reconstitution or mixing |

### 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.

COBAS, COBAS C, COBAS INTEGRA and PRECISET are trademarks of Roche.  
 All other product names and trademarks are the property of their respective owners.  
 Significant additions or changes are indicated by a change bar in the margin.  
 © 2014, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
 www.roche.com

Distribution in USA by:  
 Roche Diagnostics, Indianapolis, IN  
 US Customer Technical Support 1-800-428-2336

