

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k071644

**B. Purpose for Submission:**

New device

**C. Measurand:**

Phenobarbital

**D. Type of Test:**

Quantitative enzyme immunoassay

**E. Applicant:**

Roche Diagnostics Corp.

**F. Proprietary and Established Names:**

Online TDM Phenobarbital Assay

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.3660 Phenobarbital test system

2. Classification:

Class II

3. Product code:

DLZ

4. Panel:

91 (Toxicology)

## **H. Intended Use:**

1. Intended use(s):

See indications for use statement below.

2. Indication(s) for use:

The ONLINE TDM Phenobarbital assay is for the quantitative determination of phenobarbital in human serum or plasma on Roche automated clinical chemistry analyzers. Measurements obtained by this device are used in the diagnosis and treatment of phenobarbital use or overdose and in monitoring levels of phenobarbital.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

For Roche/Hitachi 912/917/ and MODULAR P analyzers. The cobas (lower case) is associated with Hitachi instruments, and COBAS (capital letters) is associated with COBAS Integra analyzers.

## **I. Device Description:**

The ONLINE TDM Phenobarbital assay is comprised of two ready to use reagents. Reagent 1 is a conjugate reagent consisting of phenobarbital conjugate, piperazine-N, bugger, preservative and stabilizer. Reagent 2 is a latex antibody reagent consisting of anti-phenobarbital antibody (mouse monoclonal), latex microparticle, MOPS, bugger, stabilizer and preservative. The previously cleared preciset TDM calibrators are not included with the device but are suggested in the package insert (k031856).

## **J. Substantial Equivalence Information:**

1. Predicate device name(s):

Roche COBAS INTEGRA Phenobarbital assay

2. Predicate K number(s):

k951595

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	Quantitative measurement of phenobarbital	Same
Matrix	Serum or plasma	Same
Storage	2-8 C	2-8 C

Differences		
Item	Device	Predicate
Measuring range	2.4 – 60 µg/mL	0.6-60 µg/mL
Methodology	Homogeneous enzyme immunoassay	Fluorescence polarization
Instrumentation	Roche/Hitachi 912, 917 and Modular P	Roche COBAS Integra

**K. Standard/Guidance Document Referenced (if applicable):**

Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy <http://www.fda.gov/cdrh/oivd/guidance/950.html>

**L. 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.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

All of the performance characteristics for this submission were conducted on the Roche Hitachi 917.

*a. Precision/Reproducibility:*

Within-run precision was conducted by running three levels of control (low, mid and high controls) and two levels of human serum pools (low and high) 21 times on the Hitachi 917. The sponsor acceptance criteria for an observed within run precision no greater than a standard deviation of 0.75, up to a concentration of 15 mg/mL, or CV of less than 5% at higher concentrations.

Material	TDM I	TDM II	TDM III	HSP 1	HSP 2
Concentration	9.13	23.30	44.40	~15	~35
Mean	9.80	24.34	45.21	15.66	38.18
SD	0.14	0.33	0.27	0.15	0.34
CV%	1.4	1.4	0.6	0.9	0.9
Min	9.64	23.90	44.68	15.40	37.33
Max	10.24	25.23	45.74	15.94	38.87

Between-day precision was assessed conducted by running three levels of control (low, mid and high controls) and two levels of human serum pools (low and high) for 21 days on the Hitachi 917. The sponsor acceptance criteria was that the within run precision observed should be no greater than a standard deviation of 0.9, up to a concentration of 15 µg/mL, or CV less than 7% at higher concentration. The total and between-day run precision observed should be no greater than a standard deviation of 0.9, up to a concentration of 15 µg/mL, or CV less than 7% at higher concentration.

Specimen	TDM I	TDM II	TDM III	HSP 1	HSP 2
Total Mean	9.62	24.09	45.24	15.39	37.59
Within Run Imprecision SD	0.125	0.180	0.369	0.149	0.336
Within Run Imprecision CV%	1.3	0.7	0.8	1.0	0.9
Total Imprecision SD	0.338	0.586	0.822	0.432	0.760
Total Imprecision CV%	3.5	2.4	1.8	2.8	2.0
Between-day Imprecision SD	0.315	0.558	0.735	0.406	0.682
Between-day Imprecision CV%	3.3	2.3	1.6	2.6	1.8

*b. Linearity/assay reportable range:*

Linearity was assessed via an 11-level dilution series that were prepared using a phenobarbital spiked human serum pool diluted with a non-spiked serum pool on the Hitachi 917. Recovery was determined by comparison of the measured value to the theoretical value. The theoretical values were calculated according to the dilution factors. The sponsor's acceptance criteria were recovery within +/-10% recovery or less than 1.7 µg/mL difference between the observed and theoretical concentrations up to 60 µg/mL. The samples were measured in triplicate. The recoveries for the lower assay range

were 89.39% to 113.75% for samples at 2.44 µg/mL to 2.67 µg/mL. The recoveries were 91.3% to 104.8% from for samples ranging from 7.5 µg/mL to 72.9 µg/mL.

The sponsor conducted a sample dilution study with the calibrator A diluent that is recommended in the package insert. The samples (55.95- 10.97 ug/mL) were diluted in a 1:1 ratio and the diluted sample value was multiplied by two and compared to the pre-diluted sample value. The recovery results ranged from 95 to 104%. The assay range for the ONLINE TDM phenobarbital assay is 2.4 - 60 µg/mL. The sponsor's recovery acceptance criterion is +/- 10%. The package insert instructs the users to manually dilute samples that fall outside the assay range.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

The already cleared Preciset TDM I Calibrators (k031856) are prepared to contain known quantities of phenobarbital in normal human serum and are traceable to USP reference standards. These calibrators are used to establish a standard curve from which the quantity of unknown specimens can be determined. The previously cleared Preciset TDM calibrators are not included with the device but are suggested in the package insert.

*d. Detection limit:*

To determine the lower detection limit (LDL), twenty one replicates of the zero calibrator and five replicates of calibrator b (5.0 µg/mL) were assayed in a single run on the Hitachi 917. The observed LDL with zero calibrator is 0.15 µg/mL at 2 SD. The LDL was within the sponsor's acceptable limits of less than 1.7 µg/mL.

To determine the functional sensitivity, 7 clinical samples having concentrations between zero and twice the LDL were assayed in triplicate daily for ten days on the same instrument on the Hitachi 917. The acceptance criterion for functional sensitivity was calculated as the lowest concentration from clinical samples with a CV of less than 20%. The observed functional sensitivity on the Hitachi 917 is 1.44 µg/mL.

*e. Analytical specificity:*

The sponsor tested common substances and biological materials for interference with the phenobarbital assay at a concentration of 15 µg/mL in drug positive samples on the Hitachi 917. Spiked samples containing the interferent substances were run in triplicates in a recovery study. The sponsor states that there were no deviations from the expected results and all drug interferences passed with 10% of the referenced values.

Cross-reactivity was assessed by spiking cross-reactants into serum pools that contained approximately 15 µg/mL phenobarbital. The sponsor definitions are:

Cross-reactivity equation	<p>If Da-Dt &lt; LDL claim, then ND (Not Detectable) If Da-Dt &gt; LDL claim, then the cross-reactivity is calculated as follows:</p> $\% \text{ Cross-Reactivity} = [(Da-Dt) / C] \times 100$ <p>Dt = Concentration of Control Analyte Spike in serum  Da = Concentration of (Analyte + Cross-Reactant)  C = Concentration of Cross-Reactant</p>
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Cross reactivity was ND for all of the following compounds except Butabarbital (0.15%), Mephobarbital (0.18%), Secobarbital (0.15%) and Butalbital (0.67%) and are placed in the package insert.

Compounds	Concentration Tested	% Cross-reactivity
Amobarbital	1000	ND
Aprobarbital	1000	ND
Butabarbital	1000	0.15
5,5 Diallylbarbituric acid	1000	ND
Mephobarbital	1000	0.18
Secobarbital	1000	0.15
Acetylsalicylic acid	1000	ND
Amitriptyline	9	ND
Barbital	1000	ND
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
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
Mephentoin	1000	ND
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-ethylmalon- amide (PEMA)	1000	ND
Phenytoin	1000	ND
P-Hydroxyphenobarbital	200	ND
Primidone	120	ND
Promethazine	0.23	ND
Theophylline	200	ND
Thiopental -Na	1000	ND
Valproic acid	1000	ND

The sponsor also conducted an interference study to evaluate the effects of bilirubin, hemolysis, lipemia, triglycerides, total protein, HAMA, heterophilic antibodies and rheumatoid factor with their phenobarbital assay on the Hitachi 917. The sponsor claims no interference with either assay as their recovery results met their acceptance criteria of +/- 10% of the initial value. There was no significant interference for conjugated and unconjugated bilirubin up to 66 mg/dL, hemolysis up to 1000 mg/dL, lipemia up to 600, triglycerides up to 1000 mg/dL, proteins between 2-14 g/dL, no HAMA interference and rheumatoid factors up to 200 IU/mL.

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

The ONLINE TDM Phenobarbital assay for the Roche/Hitachi 917 analyzer (y) was compared to the COBAS FP Phenobarbital on the BOBAS Integra 700 analyzer (x). Fifty-three non pooled human samples that ranged from 3.0-52.4 ug/mL were assayed and Passing-Bablok and linear regression was calculated and the results are located below.

	Passing Bablok	Linear Regression
Slope	1.042	1.047
Intercept	-0.215	-0.339
Correlation	Tau= 0.955	R=0.996

b. *Matrix comparison:*

Plasma pairs were obtained from an in-house blood draw and samples processed according to labeling requirements for each tube. All individuals donating were Phenobarbital and related analyte free. The recoveries for ½ K2 EDTA, K2 EDTA, K2 EDTA ½, ½ K3 EDTA, K3 EDTA, K3 EDTA ½, ½ Lithium Heparin, Lithium Heparin, Lithium Heparin ½, Lithium, ½ Sodium Heparin, Sodium Heparin, Sodium heparin ½ and its serum equivalent were calculated and the results are shown in the table below.

Anticoagulant	Sample Range (µg/mL)	n	Recovery range %
½ K2 EDTA	4.18-54.25	13	94- 105
K2 EDTA	4.18-60.54	47	90-101
½ K3 EDTA	10.46-54.07	13	95-105
K3 EDTA	4.18-60.54	49	91-105
½ Lithium Heparin	13.38-60.54	13	96-103
Lithium Heparin	13.38-60.54	10	97-103
Lithium	20.9-59.3	7	96-109
½ Sodium Heparin	7.76-58.79	14	90-110
Sodium Heparin	7.76-58.76	19	93-107

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable.

b. *Clinical specificity:*

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The sponsor has referenced the following expected values in the package insert.

“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 ug/mL. Variation in metabolism and absorption of the drug may cause levels to rise above 40 µg/mL or fall below 15 µg/mL. The most frequent dose-related side effect is sedation, to which a tolerance usually develops. Phenobarbital serum levels above 40 µg/mL are often associated with nystagmus, ataxia and dysarthria. 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.”

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

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

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.