

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

A. 510(k) Number:

k081231

B. Purpose for Submission:

New assay

C. Measurand:

Phenobarbital

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Abbott Laboratories

F. Proprietary and Established Names:

ARCHITECT *i*Phenobarbital Assay

ARCHITECT *i*Phenobarbital Calibrators (A-F)

G. Regulatory Information:

1. Regulation section:

21 CFR §862.3660, Phenobarbital test system

21 CFR §862.3200, Calibrator

2. Classification:

Class II

3. Product code:

DLZ – Enzyme immunoassay, phenobarbital

DLJ – Calibrator, drug specific

4. Panel:

91 (Toxicology)

H. Intended Use:

1. Intended use(s):

See indications for use statement below.

2. Indication(s) for use:

Reagents:

The ARCHITECT *i*Phenobarbital assay is an in vitro chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of phenobarbital, an anticonvulsant and sedative-hypnotic drug, in human serum or plasma on the ARCHITECT *i* System with STAT protocol capability. The measurements obtained are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of phenobarbital to help ensure appropriate therapy.

Calibrators:

The ARCHITECT *i*Phenobarbital Calibrators are for the calibration of the ARCHITECT *i* System with STAT protocol capability when used for the quantitative determination of phenobarbital in human serum or plasma.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

For use on Abbott ARCHITECT *i*1000SR and *i*2000 SR instruments

I. Device Description:

The ARCHITECT *i*Phenobarbital Reagent Kit is comprised of two ready to use solutions. The bottle bearing the “Microparticles Symbol” contains mouse monoclonal anti-phenobarbital antibodies coated microparticles in TRIS buffer, protein stabilizer and preservative. The bottle bearing the “Conjugate Symbol” contains phenobarbital acridinium-labeled conjugate in MES buffer, protein stabilizer and preservative.

ARCHITECT *i* instruments with STAT protocol capability (*i*X000SR) allow for random and continuous access as well as priority and automated reset capability; there is no difference in assay protocol in the STAT mode. The *i*Phenobarbital assay is only indicated for use on *i*1000SR and *i*2000 SR instruments.

The ARCHITECT *i*Phenobarbital Calibrator Kit consists of 6 bottles which contain human serum nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HCV, and anti-HIV-1/HIV-2, sodium azide, and different amounts of phenobarbital.

J. Substantial Equivalence Information:

1. Predicate device name(s):

AxSYM Phenobarbital

2. Predicate K number(s):

k940596

3. Comparison with predicate:

Reagent Similarities:

| Characteristics | Device | Predicate |
|-----------------|---|--|
| Product Type | Immunoassay | Immunoassay |
| Intended Use | The ARCHITECT <i>i</i> Phenobarbital assay is an <i>in vitro</i> chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of phenobarbital, an anticonvulsant and sedative-hypnotic drug, in human serum or plasma on the ARCHITECT <i>i</i> System with <i>STAT</i> protocol capability. The measurements obtained are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of phenobarbital to help ensure appropriate therapy. | The AxSYM Phenobarbital assay is a reagent system for the quantitative measurement of phenobarbital, an anti-convulsant and sedative-hypnotic drug, in serum or plasma. The measurements obtained are used in the diagnosis and treatment of phenobarbital overdose and in monitoring levels of phenobarbital to ensure appropriate therapy. |
| Measuring Range | 1.10 µg/mL – 80.00 µg/mL | 1.10 µg/mL – 80.00 µg/mL |
| Specimen Type | Serum or Plasma (collected in lithium heparin, potassium EDTA, sodium EDTA, potassium oxalate and sodium heparin tubes) | Serum or Plasma (collected in heparin, citrate, EDTA or oxalate collection tubes) |
| Storage | 2 – 8 °C | 2 – 8 °C |

Reagent Differences:

| Characteristics | Device | Predicate |
|-----------------|---|---|
| Platform | ARCHITECT <i>i</i> System | AxSYM System |
| Components | <p>Microparticles - 1 Bottle (6.6 mL) Anti-phenobarbital (mouse, monoclonal) coated goat anti-mouse (GAM) microparticles in TRIS buffer with protein (bovine) stabilizer. Preservative: ProClin 300.</p> <p>Conjugate - 1 Bottle (5.9 mL each) Phenobarbital acridinium-labeled conjugate in MES buffer with surfactant. Preservative: ProClin 300.</p> | <p>1 bottle (14.5mL) <25% Phenobarbital Antiserum (Sheep, Polyclonal) in normal saline with protein stabilizers. Preservative: Sodium Azide (Reagent Bottle 1)</p> <p>1 bottle (8.6 mL) Pretreatment Solution. Surfactant in TRIS buffer. Preservative: Sodium Azide. (Reagent Bottle 2)</p> |

| | | |
|-------------------------|---|---|
| | | 1 bottle (15.1 mL) <0.01% Phenobarbital Fluorescein Tracer in TRIS buffer containing surfactant. Preservative: Sodium Azide. (Reagent Bottle 3) |
| Immunoassay Methodology | Chemiluminescent Microparticle Immunoassay (CMIA) | Fluorescence Polarization Immunoassay (FPIA) |

Calibrator Similarities:

| Characteristics | Device | Predicate |
|------------------------------|--|--|
| Intended Use | The ARCHITECT <i>i</i> Phenobarbital Calibrators are for the calibration of the ARCHITECT <i>i</i> System with <i>STAT</i> protocol capability when used for the quantitative determination of phenobarbital in human serum or plasma. | The AxSYM Phenobarbital Standard Calibrators are for the standard calibration of the AxSYM System when used for the quantitative measurement of phenobarbital in human serum or plasma. |
| Standardization/Traceability | Internal Reference Calibrators are manufactured gravimetrically using USP Reference Standard Phenobarbital. The ARCHITECT <i>i</i> Phenobarbital Calibrators are matched to the Internal Reference Calibrators. | Abbott manufactures internal reference standards using Phenobarbital (USP Reference Standard). Phenobarbital calibrators are manufactured gravimetrically and tested against these internal reference standards. |
| Calibrator Levels | 6 levels | 6 levels |
| Matrix | Human serum | Human serum |

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

CLSI EP5-A2: *Evaluation of Precision Performance of Clinical Chemistry Devices*
CLSI EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples*
CLSI EP7-A2: *Interference Testing in Clinical Chemistry; Approved Guideline*
CLSI EP17-A: *Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*

L. Test Principle:

Sample, anti-phenobarbital antibody coated paramagnetic microparticles, and phenobarbital acridinium labeled conjugate are combined to create a reaction mixture. The anti-phenobarbital antibody coated microparticles bind to phenobarbital present in the sample and to the phenobarbital acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are added to the reaction mixture. The resulting

chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of phenobarbital in the sample and the RLUs detected by the ARCHITECT *i* System optics

M. Performance Characteristics (if/when applicable):

Performance was established on the ARCHITECT *i*2000_{SR}. The sponsor also provided data that demonstrated equivalent performance on the ARCHITECT *i*1000_{SR}. The data summarized below is the data generated on the ARCHITECT *i*2000_{SR}.

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision studies were performed on three ARCHITECT *i*2000SR instruments, each using a different lot of reagent and calibrators. The calibration curve generated for each reagent lot was performed on each instrument by running the calibrators in replicates of two. The calibration curve generated for each reagent lot was stored on each instrument for the duration of the study.

The assay was run twice a day for 20 days using three levels of Abbott Immunoassay-MCC (Liquid) and three levels of patient serum in replicates of two, resulting in a total of 80 replicates for each instrument/lot control and panel:

ARCHITECT *i*Phenobarbital: Precision

| Sample | Instrument/ Reagent Lot | Mean (ug/mL) | Within Run | | Total | |
|---------|----------------------------|-----------------|------------|------|-------|------|
| | | | SD | %CV | SD | %CV |
| Level 1 | 1 | 9.37 | 0.31 | 3.31 | 0.34 | 3.63 |
| | 2 | 9.26 | 0.32 | 3.46 | 0.36 | 3.89 |
| | 3 | 9.40 | 0.24 | 2.55 | 0.33 | 3.51 |
| Level 2 | 1 | 23.59 | 0.72 | 3.05 | 0.82 | 3.48 |
| | 2 | 23.34 | 0.73 | 3.13 | 1.01 | 4.33 |
| | 3 | 24.11 | 0.63 | 2.61 | 0.70 | 2.90 |
| Level 3 | 1 | 47.30 | 1.21 | 2.56 | 1.52 | 3.21 |
| | 2 | 48.51 | 1.38 | 2.84 | 1.48 | 3.05 |
| | 3 | 48.87 | 1.28 | 2.62 | 1.45 | 2.97 |
| Serum 1 | 1 | 9.64 | 0.32 | 3.32 | 0.33 | 3.42 |
| | 2 | 9.46 | 0.27 | 2.85 | 0.35 | 3.70 |
| | 3 | 9.68 | 0.27 | 2.79 | 0.32 | 3.31 |
| Serum 2 | 1 | 37.15 | 0.97 | 2.61 | 1.22 | 3.28 |
| | 2 | 37.36 | 1.06 | 2.84 | 1.41 | 3.77 |
| | 3 | 38.75 | 1.13 | 2.92 | 1.28 | 3.30 |
| Serum 3 | 1 | 56.21 | 1.52 | 2.70 | 2.09 | 3.72 |
| | 2 | 57.76 | 2.44 | 4.22 | 2.65 | 4.59 |
| | 3 | 57.32 | 1.58 | 2.76 | 2.00 | 3.49 |

Two additional studies were performed to evaluate precision at the extremes of the assay measuring range. Both the upper end and lower end of the

measurement range was evaluated with an additional 5-day precision study performed on three ARCHITECT i2000s instruments using three lots of reagents and one lot of calibrators. For the upper end study, a total of 20 replicates for each of the three reagent/instrument combinations were generated. The assay was run twice a day for five days. High values targeted a concentration above 70 µg/mL using spiked patient samples. For the low end study each of 4 samples was tested over 5 days, 2 runs per day and 10 reps per run for a total of 100 reps with each of the three reagent lots.

ARCHITECT iPhenobarbital: High End Precision

| Instrument/ Reagent Lot | n | Mean (µg/mL) | Within Run | | Total | |
|----------------------------|----|-----------------|------------|------|-------|------|
| | | | SD | %CV | SD | %CV |
| 1 | 20 | 76.98 | 1.40 | 1.82 | 1.93 | 2.51 |
| 2 | 20 | 71.18 | 2.18 | 3.06 | 2.93 | 4.11 |
| 3 | 20 | 75.07 | 3.03 | 4.04 | 3.09 | 4.12 |

ARCHITECT iPhenobarbital: Precision Very Low Concentrations

| | Sample1 | Sample2 | Sample3 | Sample 4 |
|-----------------------|---------|---------|---------|----------|
| Lot 1 Mean (µg/mL) | 1.44 | 1.87 | 2.73 | 3.56 |
| Total CV | 8.6% | 7.2% | 6.9% | 4.9% |
| Lot 2 Mean (µg/mL) | 1.27 | 1.72 | 2.61 | 3.45 |
| Total CV | 13.0% | 10.3% | 8.0% | 7.6% |
| Lot 3 Mean (µg/mL) | 1.11 | 1.55 | 2.39 | 3.26 |
| Total CV | 11.5% | 10.6% | 8.6% | 7.2% |

b. Linearity/assay reportable range:

The claimed assay range is 1.1 µg/mL – 80.0 µg/mL. Linearity within this range was assessed by diluting five spiked serum and five spiked plasma samples via an 11-level dilution series. Regression analysis of each dilution series showed that the samples were linear. (Slope range = 0.98x to 1.03x, intercept range = 0.14 to 0.56, $R^2 \geq 0.998$)

Recovery of these samples was determined by comparison of the measured value to the theoretical value calculated according to the dilution factors. The

recovery for all serum samples was 96.2 – 109.4%; the recovery for all plasma samples was 101.1 – 130.7%. Recoveries $\geq 110\%$ were in the range between 1.1 and 5 $\mu\text{g/mL}$. Absolute recoveries in this lower assay range differed from expected values by: 0.13 – 0.2 $\mu\text{g/mL}$ for serum samples and 0.04 – 0.31 $\mu\text{g/mL}$ for plasma samples.

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

The ARCHITECT iPhenobarbital Calibrators B – F are traceable to the Internal Reference Calibrators. Internal Reference Calibrators are manufactured gravimetrically using USP Reference Standard Phenobarbital. Calibrators A-F contain human plasma non-reactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HCV, and anti-HIV-1/HIV-2.

Real time and accelerated stability testing support a claim that the reagents and calibrators are stable for 12 months from manufacture when stored at 2 – 8°C. Opened reagent and calibrator may be stored at 2 – 8°C for up to 30 days.

The sponsor references a published reference for instructions on sample stability under different conditions.

d. *Detection limit:*

The limit of blank (LoB) and the limit of detection (LoD) were determined using three instruments, three lots of reagent, and one control lot. Testing consisted of 20 replicates of the A calibrator for each instrument and lot combination (total n = 180) and 15 replicates each of five low level phenobarbital samples. Calculations were performed according to CLSI EP17-A. The LoB and LoD values were below the claimed lower limit of the assay range, 1.1 $\mu\text{g/mL}$.

e. *Analytical specificity:*

Endogenous Substances: Serum samples spiked with 15 $\mu\text{g/mL}$ or 40 $\mu\text{g/mL}$ phenobarbital were supplemented with the below interfering compounds. Each test and control sample were run in replicates of 5 and the mean interference was calculated as above:

ARCHITECT iPhenobarbital: Endogenous Interferents

| Interferent | Concentration | Mean % Recovery | |
|---------------|---------------|---------------------|---------------------|
| | | 15 $\mu\text{g/mL}$ | 40 $\mu\text{g/mL}$ |
| Bilirubin | 15 mg/dL | 100.3 | 96.9 |
| Hemoglobin | 500 mg/dL | 98.3 | 97.2 |
| Protein | 3 g/dL | 94.7 | 92.8 |
| Protein | 10 g/dL | 95.0 | 94.5 |
| Triglycerides | 2500 mg/dL | 101.2 | 100.4 |

Five specimens positive for Rheumatoid Factor (RF) and five specimens positive for human anti-mouse antibodies (HAMA) were also evaluated for interference by spiking them with 15 µg/mL or 40 µg/mL phenobarbital and determining their recovery relative to unspiked samples. The mean percent recovery for the RF samples ranged from 99.3% to 99.6%; the mean percent recovery for the HAMA samples ranged from 97.0% to 100.1%.

Exogenous Compounds: For all cross-reactants tested, normal human serum (NHS) samples were used. These serum samples were spiked with phenobarbital to target concentrations at 15 and 40 µg/mL and cross-reactant. The test and control spiked samples were run in replicates of five. Percent Cross Reactivity was calculated as (Measured Value with Cross-Reactant) – (Measured Value Control) * 100 Amount of Cross-Reactant. The mean % Cross Reactivity and the grand mean % Cross Reactivity were also calculated.

ARCHITECT iPhenobarbital: Cross Reactivity

| Cross-reactant | Test Conc (µg/mL) | % Cross Reactivity – 15 µg/mL Phenobarbital | % Cross Reactivity – 40 µg/mL Phenobarbital |
|-----------------------------|--------------------------|--|--|
| Amitriptyline | 25 | -0.5 | 0.8 |
| Amobarbital | 30 | 22.1 | 21.6 |
| Aprobarbital | 100 | 1.9 | 2.6 |
| Barbital | 100 | 0.1 | 0.3 |
| Butabarbital | 100 | 1.3 | 1.6 |
| Carbamazepine-10,11-epoxide | 240 | 0.1 | -0.1 |
| Chlordiazepoxide | 100 | 0 | -0.4 |
| Chlorpromazine | 100 | 0.4 | 0.7 |
| Chlorazepate | 100 | 0.2 | 0.1 |
| Ethotoin | 300 | -0.1 | 0.1 |
| 5-Ethyl-5-phenylhydantoin | 200 | 0.4 | 0.6 |
| p-Hydroxyphenobarbital | 22 | 1.4 | 3.1 |
| Imipramine | 20 | 2.4 | 1.5 |
| Mephobarbital | 15 | 222.8 | 240.8 |
| Methsuximide | 150 | 0.2 | 0.3 |
| Pentobarbital | 100 | 1.2 | 1.5 |
| Phenytoin | 300 | 0 | -0.1 |
| Primidone | 200 | 0.4 | 0.4 |
| Secobarbital | 25 | 5.9 | 5.1 |
| Thiopental | 100 | 0.3 | -0.1 |

Amobarbital and mephobarbital (Meberal) are structurally similar to phenobarbital and may interfere with the ARCHITECT iPhenobarbital assay. The package insert contains a warning to this effect.

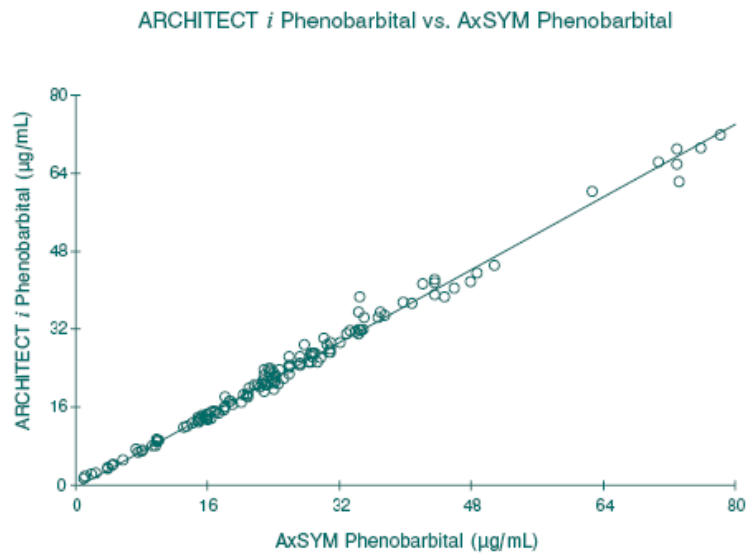
f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The ARCHITECT iPhenobarbital assay for the ARCHITECT i2000s was compared to the Roche AxSYM Phenobarbital assay by testing 132 frozen serum samples. The sample range was 1.42 to 71.65 µg/mL with the ARCHITECT iPhenobarbital assay and from 1.10 to 78.25 µg/mL with the AxSYM Phenobarbital assay. Passing-Bablok regression analysis results were:

| n = | Slope (95% CI) | Intercept (95% CI) | Correlation |
|-----|-----------------------|--------------------------|-------------|
| 132 | 0.93 (0.91 – 0.95) | -0.44 (-0.80 – -0.18) | >0.999 |



Bias analysis:

A bias analysis of ARCHITECT iPhenobarbital vs. AxSYM Phenobarbital was performed on the same 132 specimens in the range of 1.42 µg/mL to 71.65 µg/mL and 1.10 µg/mL to 78.25 µg/mL, respectively. The average bias exhibited by ARCHITECT vs. AxSYM in this study was -8.1% (95% CI -23.9 - 7.7%). Within the typical therapeutic range of phenobarbital therapy (10 to 40 µg/mL, as read in the AxSYM), the average bias was -8.7% (95% CI -18.9 - 1.5%). The sponsor has added the following to the package insert:

CAUTION: Values obtained with different assay methods should not be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended. Each user should verify their own Expected Values range based on clinical experience.

b. Matrix comparison:

The suitability of the specimen collection tubes in the table below were evaluated using 20 spiked blood samples spanning the assay range. Samples were aliquoted into each type of tube and tested in triplicate. Serum (no additives) was used as the control. Recovery was calculated and the results are shown in the table below:

ARCHITECT iPhenobarbital: Matrix Comparison

| Anticoagulant | Mean recovery (%) |
|-------------------|-------------------|
| 2K-EDTA | 100 |
| 3K-EDTA | 96 |
| 2Na-EDTA | 100 |
| Potassium Oxalate | 96 |
| Sodium Heparin | 98 |
| Lithium Heparin | 100 |

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:

Strong correlations have been shown between serum levels of phenobarbital and both therapeutic effect and toxicity. Clinical observations indicate that toxicity of phenobarbital is increased in patients with renal disease. Phenobarbital toxicity primarily affects the central nervous system. Toxic levels can lead to nystagmus, vertigo, and ataxia. A small number of patients develop hypersensitivity to the drug. Some

patients under chronic treatment develop macrocytosis and megablastic anemia as well as osteomalacia. Most patients will receive maximum seizure control when serum levels of phenobarbital are in the range of 15 – 40 µg/mL.

CAUTION: Values obtained with different assay methods should not be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended. Each user should verify their own Expected Values range based on clinical experience.

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.