

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

A. 510(k) Number:

k080696

B. Purpose for Submission:

New device

C. Measurand:

Phenytoin

D. Type of Test:

Quantitative chemiluminescent microparticle immunoassay

E. Applicant:

Abbott Laboratories

F. Proprietary and Established Names:

Architect iPhenytoin Immunoassay
Architect iPhenytoin Calibrators (A-F)

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
<u>DIP</u>	<u>II</u>	<u>862.3350</u>	<u>Toxicology (91)</u>
<u>DLJ</u>	<u>II</u>	<u>862.3200</u>	<u>Toxicology (91)</u>

H. Intended Use:

1. Intended use(s):

See Indications for use below.

2. Indication(s) for use:

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

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

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

The ARCHITECT iPhenytoin reagents and calibrators are designed to be used in the ARCHITECT *i* System platform.

I. Device Description:

The device is supplied as ready-to-use, two-reagent kit. Microparticles reagent bottle 1 (6.6 mL) contains anti-phenytoin (mouse, monoclonal) coated goat anti-mouse (GAM) microparticles in TRIS buffer with protein (bovine) stabilizer and preservative: ProClin 300. Reagent bottle 2 (5.9 mL) contains Phenytoin acridinium-

labeled conjugate in MES buffer with surfactant. Minimum concentration: 6 ng/mL. Preservative: ProClin 300.

The calibrators consisting of six human serum based materials at approximate concentrations of 0, 2.5, 5.0, 10.0, 20.0 and 40.0 are sold separately. The product is tested and found to be nonreactive for HBsAg, HIV-1 Ag or HIV-1 RNA, anti-HCV, and anti-HIV-1/HIV-2. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with caution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Abbott AxSYM Phenytoin
2. Predicate 510(k) number(s):
k935375
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use (Reagent)	Quantitative measurement of phenytoin used in monitoring levels of phenytoin to help ensure appropriate therapy.	Same
Where Used	Clinical Laboratories	Same
Assay Protocol	Competitive assay	Same
Interpretation of Results	Standard Curve	Same
Measuring Range	0.5-40.0 ug/mL	Same
Intended Use (Calibrators)	Calibration of a phenytoin assay	Same
Number of Calibrators	Six	Same
Traceability	USP Reference Material	Same

Differences		
Item	Device	Predicate
Platform	Abbott Architect	Abbott AxSym
Methodology	Chemiluminescent Microparticle Immunoassay (CMIA)	Fluorescence Polarization Immunoassay (FPIA)
Matrix	MES buffer and stabilizers	Dextrose buffer and stabilizers

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

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Second Edition
- CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI EP17-A: Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.
- CLSI EP7-A2: Interference Testing in Clinical Chemistry: Approved Guideline-Second Edition

L. Test Principle:

The assay is intended for the quantitative measurement of phenytoin in human serum or plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology. The patient sample, anti-phenytoin coated paramagnetic microparticles, and phenytoin acridinium-labeled conjugate are combined to create a reaction mixture. The anti-phenytoin coated microparticles bind to phenytoin present in the sample and to the phenytoin 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 phenytoin in the sample and the RLUs detected by the ARCHITECT *i* System optics.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Following CLSI EP5-A2, the sponsor evaluated the precision using three reagents and three levels of Multi-constituent Controls (MCC) Level 1, Level 2, and Level 3 with concentrations at approximately 7, 14, and 25 ug/mL) and three serum panels prepared by adding phenytoin into a pool of human sera at approximately 10, 20, and 30 ug/mL concentrations of phenytoin. Precision tests were run on three ARCHITECT *i* systems using three lots of reagents and one lot of calibrators. Studies were carried out in duplicates of two runs per day over 20 days (total of 80 data points). The results are tabulated below.

Sample	Instrument/ Reagent Lot	n	Mean (µg/mL)	Within Run SD %CV		Total SD %CV	
Level 1	1/1	80	6.75	0.18	2.67	0.19	2.81
	2/2	80	6.91	0.20	2.89	0.21	3.04
	3/3	80	6.92	0.19	2.75	0.20	2.89

Sample	Instrument/ Reagent Lot	n	Mean (µg/mL)	Within Run		Total	
				SD	%CV	SD	%CV
Level 2	1/1	80	13.66	0.34	2.49	0.35	2.56
	2/2	80	14.25	0.35	2.46	0.40	2.81
	3/3	80	13.99	0.37	2.64	0.43	3.07
Level 3	1/1	80	24.11	0.63	2.61	0.72	2.99
	2/2	80	24.16	0.53	2.19	0.87	3.60
	3/3	80	24.09	0.76	3.15	0.82	3.40
Panel 1	1/1	80	9.74	0.26	2.67	0.28	2.87
	2/2	80	10.13	0.21	2.07	0.29	2.86
	3/3	80	10.00	0.23	2.30	0.30	3.00
Panel 2	1/1	80	19.60	0.56	2.86	0.58	2.96
	2/2	80	20.00	0.48	2.40	0.65	3.25
	3/3	80	19.87	0.55	2.77	0.65	3.27
Panel 3	1/1	80	31.10	1.06	3.41	1.16	3.73
	2/2	80	29.58	0.56	1.89	1.10	3.72
	3/3	80	30.27	0.91	3.01	1.34	4.43

A second five day precision study was performed to evaluate the upper end of the measurement range (40 µg/mL) with a sample at approximately 37 µg/mL phenytoin. This study was performed on three ARCHITECT *i* 2000SR instruments using three lots of reagents and one lot of calibrators. A total of 20 replicates for each of the three reagent/instrument combinations were generated. The results are tabulated below.

Sample	Instrument/ Reagent Lot	n	Mean (µg/mL)	Within Run		Total	
				SD	%CV	SD	%CV
High Phenytoin Pool	1/1	20	36.87	1.08	2.93	1.22	3.30
	2/2	20	37.28	1.18	3.17	1.42	3.81
	3/3	20	36.22	1.11	3.06	1.27	3.52

b. Linearity/assay reportable range:

Following CLSI Document EP6-A, the sponsor conducted studies to evaluate the dilution recovery of the ARCHITECT *i*Phenytoin assay. The sponsor used five individual serum samples and five individual Potassium EDTA (frozen) samples (Phenytoin values: 32-34 µg/mL) diluted manually with the ARCHITECT *i*Phenytoin Calibrator A. Each of the ten samples was diluted to 9 test concentration levels. Testing was done using one reagent lot and running duplicates of each diluent level. The recoveries of the diluted serum samples ranged from 99% to 107% and 93% to 105% for plasma samples.

Based on the recovery data and the limit of detection described below, the sponsor established the assay measuring range for ARCHITECT iPhenytoin assay as 0.50 µg/mL – 40.0 µg/mL.

Following CLSI document EP6-A, the sponsor conducted an additional study to further evaluate the measurement range of 0.50 to 40.00 µg/mL using Five individual serum samples and 5 individual Potassium EDTA plasma samples (frozen) were each spiked with a 4 mg/mL phenytoin solution to target sample concentration values greater than 40.00 µg/mL. Each of the ten samples was diluted to 11 concentration levels. One lot of reagents was used to test neat samples in replicates of 4 and diluted samples in duplicates. Each sample was diluted using the ARCHITECT iPhenytoin Calibrator A. The mean percent recovery of the diluted serum samples ranged from 100.6% to 106.1% and the mean percent recovery of the diluted plasma samples ranged from 99.5% to 101.8%.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The calibrators are sold separately and the labeling recommends using commercially available control materials for the quality control procedure. The sponsor provided the protocols for preparation and value assignment for calibrators. The Internal Standard Calibrators are manufactured gravimetrically using purified synthetic Phenytoin from the US Pharmacopeia (USP) Reference Standard. The ARCHITECT iPhenytoin Calibrators are matched to the Internal Standard Calibrators, which consists of calibrator buffer (MES, Dextrose and ProClin 300), Phenytoin and stabilizer. Calibrator A is prepared from human serum and contains sodium azide with ProClin. The target concentrations for Calibrators B-F are 2.5, 5, 10, 20 and 40 µg/mL Phenytoin. The stability protocols and acceptance criteria were reviewed and found to be acceptable.
- d. *Detection limit:*
To determine the lower limit of the assay range, the sponsor evaluated limit of detection (LOD) and limit of blank (LOB) using CLSI document EP17-A “Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline” The sponsor used 2 instruments, 2 lots of reagents and 2 lots of calibrators for testing on a panel of 5 samples with low phenytoin concentrations and a blank sample with 0 µg/mL phenytoin. The samples with phenytoin were prepared by diluting low phenytoin serum sample with a blank sample. Three runs were performed for each reagent lot. Each run consisted of 20 replicates of a blank sample (0 µg/mL) and 5 replicates of each low level phenytoin sample for a total of 60 replicates of the blank and 15 replicates of each low level phenytoin samples. Based on the 95th percentile calculation described in the CLSI EP17-A, LOD was determined using the algorithm, $LoD = LoB [1.645 / (1 - 1 / 4 \times df)] \sigma S$. Data in the 510(k) support the claim for the lower limit of the reportable range, 0.50 µg/mL.

e. *Analytical specificity:*

Following CLSI EP7-A2 guidelines, the sponsor evaluated the effect of endogenous potential interferents on Architect *i* Phenytoin Assay. The interferents included triglycerides (2500 mg/dL), hemoglobin (500 mg/dL), low protein (3 g/dL), high protein (10 g/dL), and bilirubin (15 mg/dL). Five human serum samples with Phenytoin concentrations targeted at 10 and 20 µg/mL were used to prepare the test panel. These human serum samples were spiked with the interferent for the test sample. An equal volume of interferent diluent was spiked into the samples to prepare the control sample. Mean recovery was within 100 ± 10% of the control results for the above interferents and at the concentrations tested.

The sponsor evaluated the effect of HAMA and rheumatoid factor (RF) by testing specimens with HAMA and RF to assess their effect on the Architect *i*Phenytoin Assay. Five human serum samples positive for Phenytoin concentrations targeted at 10 and 20 ug/mL were used to prepare the interfering panel. These human serum samples were spiked with the interferent for the test sample. An equal volume of interferent diluent was spiked into the samples to prepare the control sample Mean recovery was within 100 ± 10% of the control results for HAMA and RF.

Following CLSI EP7-A2 guidelines, the sponsor conducted a study to evaluate the potential cross-reactivity of the ARCHITECT *i* Phenytoin assay when tested with structurally similar compounds. For all cross-reactants tested (except Fosphenytoin), two pools of sera, one with essentially no residual phenytoin and one with a low level of phenytoin were split into 3 different parts. Two parts were spiked with phenytoin to reach target concentrations between approximately 9.37 and 20.23 ug/mL and the third part was not spiked with phenytoin. Compounds were spiked into the samples above to prepare the test sample and an equal volume of the interferent diluent was spiked to the samples to prepare the control sample. The test and control samples were tested five times. For the Fosphenytoin testing, two frozen normal human serum containing no phenytoin were spiked with Fosphenytoin and within 10 minutes, tested on the ARCHITECT system. The sponsor defined interference as mean percent recovery greater than +/-10% of initial values at therapeutic concentrations. No interference was detected at the concentrations tested.

<u>Test Compound</u>	<u>Concentration</u>
5(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH)	5 ug/mL
5(4-hydroxyphenyl)-5-phenylhydantoin glucuronide	100 ug/mL
Oxaprozin	230 ug/mL
(+/-) Nirvanol(5-ethyl-5-phenylhydantoin	100 ug/mL
(+/-) Mephenytoin	100 ug/mL
Fosphenytoin	40 and 60 ug/mL

f. Assay cut-off
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The sponsor performed an in-house evaluation. Measurements from the Architect *i*Phenytoin were compared to the Abbott AxSYM Phenytoin. One hundred fifty-four (154) individual specimens were measured ranging from 1.45 to 39.63 µg/mL as measured on the Architect *i* System. Six samples were spiked to achieve concentrations at the upper end of the 40 µg/mL measuring range. Two samples were excluded from the analysis because they were below the 0.50 µg/mL measuring range. The analysis of data using Passing & Bablok produced a regression equation of $y = .996x - 0.266$. The following table demonstrates the correlation between the two methods.

	Slope (95% C.I)	Intercept (95% C.I)	Correlation Coefficient
154	0.996 (0.980 to 1.011)	-0.266 (0.409 to -0.139)	0.993

A bias analysis of ARCHITECT *i*Phenytoin vs. AxSYM Phenytoin was performed on the same 154 specimens in the range of 1.45 to 39.63 µg/mL and 1.44 to 35.49µg/mL respectively. The average bias exhibited by ARCHITECT vs. AxSYM in this study was -3.95%. The 95% confidence interval of that average bias is - 18.12 to 10.22%. Within the typical therapeutic range of phenytoin therapy (10 to 20 µg/mL), the average bias was -2.73% with a 95% confidence interval of -14.61 to 9.15%.

b. *Matrix comparison:*

The sponsor conducted matrix comparison studies using 20 matched serum and plasma samples prepared with seven anticoagulants (2K-EDTA, 3K-EDTA, Na-Citrate, K-Oxalate, Na-Heparin and Li-Heparin). FDA cleared blood collection tubes were used. Serum tubes without anticoagulants were used as the control. To evaluate the anticoagulant effect along the assay measuring range, each of the 20 sets of serum/plasma tubes were spiked with phenytoin to obtain five matched sets below the therapeutic range (0.5 to < 10 µg/mL), 10 matched sets to target within the therapeutic range (10 to 20 µg/mL) and 5 matched sets to target above the therapeutic range (>20 to <= 40 µg/mL). The samples were analyzed in triplicate with one lot of ARCHITECT *i* Phenytoin reagents on the ARCHITECT *i* System. Mean recovery was within 100 ± 10% vs. serum results. Results shown in the table below:

Sample type	No	Mean % recovery vs. Serum
Serum with no additive	20	Control
2K-EDTA	20	101
3K-EDTA	20	102

2Na-EDTA	20	101
K-Oxalate	20	104
Na-Heparin	20	101
Na-Citrate	20	103
Li-Heparin	20	101

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 labeling states, as supported by literature, that most patients will receive maximum seizure control when serum levels of phenytoin are in the range of 10-20 ug/mL.

1. Buchthal F, Svensmark O, Serum concentrations of diphenylhydantoin (phenytoin) and phenobarbital and their relation to therapeutic and toxic effects. *Psychiat, Neurol, Neurochir.* 1971;74:117-36

2. Kutt H. Diphenylhydantoin: relation of plasma levels to clinical control, In: Woodbury DM, Penny JK editors, anti-epileptic drugs, New York Raven Press

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.