


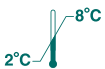


 **ARCHITECT****SYSTEM****en***i*Phenytoin**IVD** **REF** 1P34**F5-O205-2/R3**Read Highlighted Changes
Revised December, 2008

*i*Phenytoin

Customer Service**United States: 1-877-4ABBOTT****International: Call your Abbott Representative**

This package insert must be read carefully before product use. Package insert instructions must be followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	REACTION VESSELS	Reaction Vessels
IVD	<i>In Vitro</i> Diagnostic Medical Device	SAMPLE CUPS	Sample Cups
LOT	Lot Number	SEPTUM	Septum
	Expiration Date	REPLACEMENT CAPS	Replacement Caps
	Store at 2-8°C	REAGENT LOT	Reagent Lot
	Consult instructions for use	ASSAY CD-ROM	Assay CD-ROM
EC REP	Authorized Representative	CONTROL NO.	Control Number
	Manufacturer	SN	Serial Number

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

NAME

ARCHITECT *i*Phenytoin

INTENDED USE

The ARCHITECT *i*Phenytoin 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.

SUMMARY AND EXPLANATION OF TEST

Phenytoin (Dilantin) is one of the most widely prescribed anticonvulsants and is occasionally used as a myocardial antiarrhythmic. In the treatment of epilepsy, phenytoin is indicated for grand mal epilepsy (major motor) and cortical focal seizures and temporal lobe epilepsy.¹

The main pathway (about 90%) for disposition of phenytoin is by excretion of the glucuronide of para-hydroxyphenylphenyl-hydantoin (HPPH) in the urine.² It is hydroxylated in the liver and eliminated. The metabolic conversion to HPPH is a saturable process and in many cases small increments in dosage can cause a large increase in phenytoin plasma level.³ Because of the narrow therapeutic index and the wide interindividual variability in the rate of phenytoin metabolism and clearance, the determination of blood levels of phenytoin for patients receiving therapy is appropriate.⁴

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT *i*Phenytoin assay is a one-step *STAT* immunoassay for the quantitative measurement of phenytoin in human serum or plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex.

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.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests

ARCHITECT *i*Phenytoin Reagent Kit (1P34)

- **MICROPARTICLES** 1 Bottle (6.6 mL) Anti-phenytoin (mouse, monoclonal) coated goat anti-mouse (GAM) microparticles in MES buffer with protein (bovine) stabilizer. Preservative: ProClin 300.
- **CONJUGATE** 1 Bottle (5.9 mL) Phenytoin acridinium-labeled conjugate in MES buffer with surfactant. Minimum concentration: 6 ng/mL. Preservative: ProClin 300.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35 N sodium hydroxide.

ARCHITECT *i* Wash Buffer

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservative: antimicrobial agents.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials are considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens.⁵ Biosafety Level 2⁶ or other appropriate biosafety practices^{7,8} should be used for materials that contain or are suspected of containing infectious agents.

- Both components contain methylisothiazolones, which are components of ProClin, and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.




- | | |
|-----|---|
| R43 | May cause sensitization by skin contact. |
| S24 | Avoid contact with skin. |
| S35 | This material and its container must be disposed of in a safe way. |
| S37 | Wear suitable gloves. |
| S46 | If swallowed, seek medical advice immediately and show this container or label. |

- For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.
- For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between reagent kits.**
- Before loading the ARCHITECT *i*Phenytoin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
 - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
 - Over time, residual liquids may dry on the septum surface. These are typically dried salts, which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Storage Instructions

-  The ARCHITECT *i*Phenytoin Reagent Kit must be stored at 2-8°C in an upright position and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT *i*Phenytoin Reagent Kit may be stored on board the ARCHITECT *i* System with *STAT* protocol capability for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid and must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- The ARCHITECT *i*Phenytoin assay file must be installed on the ARCHITECT *i* System with *STAT* protocol capability from the ARCHITECT *i* Assay CD-ROM Addition C prior to performing the assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT *i*Phenytoin assay is µg/mL. An alternate result unit, µmol/L, may be selected for reporting results by editing assay parameter "Result concentration units" to µmol/L. The conversion formula used by the system is as follows:
Conversion Formula: (Concentration in µg/mL) x (3.96) = µmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen collection tubes listed below were verified to be used with the ARCHITECT *i*Phenytoin assay. Other specimen collection tubes, including gel separation tubes, have not been tested with this assay.

- Human serum
- Human plasma collected in:
 - lithium heparin
 - potassium EDTA
 - sodium citrate
 - sodium heparin
 - sodium EDTA
 - potassium oxalate
- Plasma samples from different anticoagulant tube types should not be used interchangeably for monitoring phenytoin. Use of citrate tubes may cause dilution effects.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the ARCHITECT *i*Phenytoin assay.

Specimen Conditions

- Do not use specimens with the following conditions:
 - heat-inactivated specimens
 - grossly hemolyzed (> 500 mg/dL)
 - obvious microbial contamination
 - cadaver specimens or any other body fluids
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged before testing if
 - they contain fibrin, red blood cells, or other particulate matter,
 - they require repeat testing, or
 - they were frozen and thawed.

Transfer clarified specimen to a sample cup or secondary tube for testing.

- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Storage

- Specimens may be stored on or off the clot or red blood cells for up to two days at room temperature (20-25°C).⁹ Specimens removed from the clot or red blood cells may be stored up to eight days refrigerated at 2-8°C.
- Serum or plasma specimens can be stored up to five months at -20°C or colder.⁹
- Avoid more than five freeze/thaw cycles.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the clot or red blood cells.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient or on wet or dry ice. Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

- 1P34 ARCHITECT *i*Phenytoin Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System with *STAT* protocol capability
- 6L81 ARCHITECT *i* **ASSAY CD-ROM** - US - Addition C
- 8K30 ARCHITECT *i* **ASSAY CD-ROM** - WW (excluding US) - Addition C
- 1P34-01 ARCHITECT *i*Phenytoin Calibrators
- 6E20-10 Abbott Immunoassay-MCC (Liquid) or other commercial controls
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM**
- ARCHITECT *i* **REPLACEMENT CAPS**
- Pipettes or pipette tips (optional)

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the ARCHITECT *i*Phenytoin Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
 - **Invert the microparticle bottle 30 times.**
 - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles remain adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
 - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
 - Once the microparticles have been resuspended, place a septum on the bottle. For instructions on placing septums on bottles refer to the **Handling Precautions** section of this package insert.
- Load the ARCHITECT *i*Phenytoin Reagent Kit on the ARCHITECT *i* System with *STAT* protocol capability.
 - Verify that all necessary assay reagents are present.
 - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
 - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.

- Order tests.
 - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- The minimum sample volume is calculated by the system and is printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
 - Priority: 70 µL for the first ARCHITECT *i*Phenytoin test plus 20 µL for each additional ARCHITECT *i*Phenytoin test from the same sample cup.
 - ≤ 3 hours on board: 150 µL for the first ARCHITECT *i*Phenytoin test plus 20 µL for each additional ARCHITECT *i*Phenytoin test from the same sample cup.
 - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare calibrators and controls.
 - ARCHITECT *i*Phenytoin Calibrators and controls should be prepared according to their respective package inserts.
 - To obtain the recommended volume requirements for the ARCHITECT *i*Phenytoin Calibrators, hold the bottles **vertically** and dispense 5 drops of each calibrator into each respective sample cup. Dispense 150 µL of each control into each respective sample cup.
- Load samples.
 - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT Operations Manual, Section 3.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with a phenytoin value exceeding 40.00 µg/mL are flagged with the code ">40.00" and may be diluted with the Manual Dilution Procedure.

- Manual dilutions should be performed as follows:
 - The suggested dilution for a phenytoin test is 1:10.
 - Add 10 µL of the patient specimen to 90 µL of ARCHITECT *i*Phenytoin Calibrator A.
 - The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution. The result (before the dilution factor is applied) should be greater than 0.50 µg/mL.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT *i*Phenytoin calibration, test calibrators A, B, C, D, E, and F in duplicate. A single sample of each phenytoin control level must be tested to evaluate the assay calibration. Ensure that assay control values are within established ranges. Calibrators should be priority loaded.
- Calibration Range: 0.0 - 40.0 µg/mL.
- Once an ARCHITECT *i*Phenytoin calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
 - A reagent kit with a new lot number is used.
 - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT *i*Phenytoin assay is that a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. **Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.**

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and must be retested. Recalibration may be indicated.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT *i*Phenytoin assay belongs to method group 2.

Use ARCHITECT *i*Phenytoin Calibrators in place of MasterCheck as described in the ARCHITECT System Operations Manual, Appendix B.

RESULTS

Calculation

The ARCHITECT *i*Phenytoin assay uses a 4 Parameter Logistic Curve Fit (4PLC, Y-weighted) data reduction method to generate a calibration curve.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measurement Range (Reportable Range)

The measurement range of the ARCHITECT *i*Phenytoin assay is 0.50 µg/mL to 40.00 µg/mL.

LIMITATIONS OF THE PROCEDURE

- If the ARCHITECT *i*Phenytoin assay results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- **Samples from patients receiving fosphenytoin should be drawn at least 2 hours following IV administration and 4 hours following IM administration according to the recommendations of the manufacturer. Phenytoin concentrations measured before complete conversion of fosphenytoin will not reflect phenytoin concentrations ultimately achieved.**¹⁰
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).^{11,12} Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.¹²
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.¹³ The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed. Additional information may be required for diagnosis.

EXPECTED VALUES

Strong correlations have been shown between phenytoin serum levels for both therapeutic and toxic effects.¹⁴ Clinical observations indicate that toxicity of phenytoin is increased in patients with renal disease.¹⁵ Phenytoin toxicity primarily affects the central nervous system. Toxic levels can lead to nystagmus, vertigo, ataxia, psychoses and even convulsions.² Chronic treatment leads to hyperplasia of gums,¹⁶ anemia¹⁷ and osteomalacia.¹⁸ The frequency and severity of dose-dependent toxic effects increases as the serum level rises above 20 µg/mL. Most patients will receive maximum seizure control when serum levels of phenytoin are in the range of 10-20 µg/mL.^{14,19}

Refer to the drug manufacturer's package insert or the Physicians' Desk Reference (PDR) for proper drug dosage and for phenytoin measurement sampling time.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT *i*Phenytoin assay is designed to have an assay precision of $\leq 10\%$ total CV.

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Protocol EP5-A2.²⁰ Abbott Immunoassay-MCC (Liquid) (Levels 1, 2, and 3) and three human serum panels were assayed using three lots of reagents in replicates of two at two separate times per day for 20 days on three instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following tables.*

Sample	Instru- ment	Reagent Lot	n	Mean ($\mu\text{g/mL}$)	SD	Within Run %CV	Total SD	Total %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
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

* Representative data; results in individual laboratories may vary from these data.

In order to assess the precision near the high end of the measurement range (40.00 $\mu\text{g/mL}$), a study was performed using three lots of reagents in replicates of two at two separate times per day for five days on three instruments. Each reagent lot used a single calibration curve throughout the study. Data from this study are summarized in the following table.*

Sample	Instru- ment	Reagent Lot	n	Mean ($\mu\text{g/mL}$)	SD	Within Run %CV	Total SD	Total %CV
High Panel	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

* Representative data; results in individual laboratories may vary from these data.

Recovery

The ARCHITECT *i*Phenytoin assay is designed to have a mean recovery of $100 \pm 10\%$.

A study was performed on five serum samples and five plasma samples, where phenytoin was spiked into the samples to target concentrations of 0, 4, 8, 20, and 30 $\mu\text{g/mL}$. The concentration of phenytoin was determined using the ARCHITECT *i*Phenytoin assay and the resulting percent recovery was calculated. The individual percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 86.1% to 101.5% and for plasma ranged from 88.1% to 97.8%.* The mean percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 91% to 94% and for plasma ranged from 90% to 95% with a grand mean for serum and plasma of 93%.*

* Representative data; results in individual laboratories may vary from these data.

Linearity

The ARCHITECT *i*Phenytoin assay is designed to have a mean recovery of $100 \pm 10\%$ of the expected results for the diluted samples.

A linearity study was performed by diluting three serum samples and three plasma samples with the ARCHITECT *i*Phenytoin Calibrator A. The concentration of phenytoin was determined using the ARCHITECT *i*Phenytoin assay and the resulting percent recovery was calculated.

The individual percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 97.4% to 109.8% and for plasma ranged from 93.0% to 105.9%.* The mean percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 100.6% to 106.1% and for plasma ranged from 99.5% to 101.8%.* Representative data from this study are summarized in the following tables.*

ARCHITECT <i>i</i> Phenytoin Serum Samples			
Specimen	Dilution Factor	Observed Concentration ($\mu\text{g/mL}$)	% Recovery ^a
1	Undiluted	32.70	–
	1:2	16.20	99
	1:6	5.31	97
	1:10	3.25	99
2	Undiluted	32.68	–
	1:2	17.63	108
	1:6	5.91	108
	1:10	3.38	103
3	Undiluted	31.44	–
	1:2	16.89	107
	1:6	5.50	105
	1:10	3.34	106

ARCHITECT <i>i</i> Phenytoin Plasma Samples			
Specimen	Dilution Factor	Observed Concentration ($\mu\text{g/mL}$)	% Recovery
1	Undiluted	33.12	–
	1:2	17.43	105
	1:6	5.64	102
	1:10	3.25	98
2	Undiluted	34.49	–
	1:2	17.52	102
	1:6	5.68	99
	1:10	3.30	96
3	Undiluted	34.13	–
	1:2	17.01	100
	1:6	5.68	100
	1:10	3.53	103

An additional study was performed to assess linearity across the measurement range (0.50 to 40.00 $\mu\text{g/mL}$). This study was performed using one lot of reagents in replicates of four on one instrument. The individual percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 92.7% to 108.1% and for plasma ranged from 89.3% to 109.6%.* The mean percent recovery of the ARCHITECT *i*Phenytoin assay for serum ranged from 99.0% to 104.7% and for plasma ranged from 96.6% to 104.2%.* Representative data from this study are summarized in the following tables.*

ARCHITECT <i>i</i> Phenytoin Serum Sample			
Specimen	Dilution Factor	Observed Concentration ($\mu\text{g/mL}$)	% Recovery
A	Undiluted	39.60	–
	1:2	21.03	106
	1:10	4.21	106
	1:40	1.02	103

ARCHITECT <i>i</i> Phenytoin Plasma Sample			
Specimen	Dilution Factor	Observed Concentration (µg/mL)	% Recovery
B	Undiluted	39.69	–
	1:2	21.02	106
	1:10	4.10	103
	1:40	0.95	96

$$^a \text{ \% Recovery} = \frac{\text{Observed Diluted Concentration (µg/mL)} \times \text{Dilution Factor}}{\text{Observed Undiluted Concentration (µg/mL)}} \times 100$$

* Representative data; results in individual laboratories may vary from these data.

Sensitivity

Sensitivity is defined as the limit of detection (LoD). The limit of blank (LoB) and LoD of the ARCHITECT *i*Phenytoin assay were determined with guidance from CLSI Protocol EP17-A²¹ using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using one blank (60 replicates) and five low level phenytoin samples (15 replicates each); LoB = 0.02 µg/mL and LoD = 0.05 µg/mL.*

* Representative data; results in individual laboratories may vary from these data.

Specificity

The specificity of the ARCHITECT *i*Phenytoin assay was determined by studying the cross-reactivity of compounds whose chemical structure or concurrent usage could cause potential interference with the ARCHITECT *i*Phenytoin assay. Specificity of the assay was determined in the absence and presence of phenytoin by spiking each compound into human serum specimens with phenytoin levels spiked between 9.37 and 20.23 µg/mL. The average amount of interference observed during the study ranged from 0% to 1.6%.*

Test Compound	Concentration (µg/mL)
5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH)	5
5-(4-hydroxyphenyl)-5-phenylhydantoin glucuronide (p-HPPH glucuronide)	100
Oxaprozin	230
+/-Mephenytoin	100
+/-Nirvanol,(5-ethyl-5-phenylhydantoin)	100

* Representative data; results in individual laboratories may vary from these data.

Other Test Compound

Specificity of the assay was determined by spiking fosphenytoin, a phosphate ester of the anti-convulsant phenytoin, into serum specimens at 40 and 60 µg/mL. The average amount of interference observed was 1.0%.*

* Representative data; results in individual laboratories may vary from these data.

Interference

Potential interference in the ARCHITECT *i*Phenytoin assay from the following compounds is designed to have a mean recovery of 100 ± 10% of the control results at the levels indicated.

A study based on guidance from the CLSI Protocol EP7-A²² was performed for the ARCHITECT *i*Phenytoin assay. Serum specimens with phenytoin levels were targeted at 10 and 20 µg/mL and supplemented with the following potentially interfering compounds. The mean percent recovery in this study ranged from 96.6 to 107.0%.*

Potentially Interfering Compound	Concentration	Percent Recovery (Individual)
Triglycerides	2500 mg/dL	95.3 - 99.5
Hemoglobin	500 mg/dL	97.7 - 102.7
Bilirubin	15 mg/dL	97.6 - 103.1
Low Protein	3 g/dL	94.4 - 108.7
High Protein	10 g/dL	97.3 - 108.9

* Representative data; results in individual laboratories may vary from these data.

Evaluation of Other Potentially Interfering Compounds

The ARCHITECT *i*Phenytoin assay is designed to have a mean recovery of 100 ± 10% in the presence of HAMA and rheumatoid factor (RF).

In a study, the ARCHITECT *i*Phenytoin assay was evaluated by testing specimens with HAMA and RF to further assess the clinical specificity. Five specimens positive for HAMA and five specimens positive for RF were evaluated for % recovery with phenytoin spiked into each specimen to target concentrations of 10 and 20 µg/mL. The individual percent recovery for HAMA specimens ranged from 95.9 to 104.6% and for RF specimens ranged from 98.3 to 112.6%.* The mean percent recovery for HAMA specimens ranged from 99.0 to 100.9% and for RF specimens ranged from 100.4 to 103.8%.*

* Representative data; results in individual laboratories may vary from these data.

Method Comparison

The ARCHITECT *i*Phenytoin assay is designed to have a slope of 1.0 ± 0.1 and a correlation coefficient (r) of ≥ 0.95 for specimens when compared to AxSYM Phenytoin. A study was performed using serum specimens and data were analyzed using the Passing-Bablok^a regression method and are summarized in the following table.*

ARCHITECT <i>i</i> Phenytoin vs. AxSYM Phenytoin			
Number of Observations	Slope (95% CI ^b)	Intercept (95% CI)	Correlation Coefficient
154	0.996 (0.980 to 1.011)	-0.266 (-0.409 to -0.139)	0.993

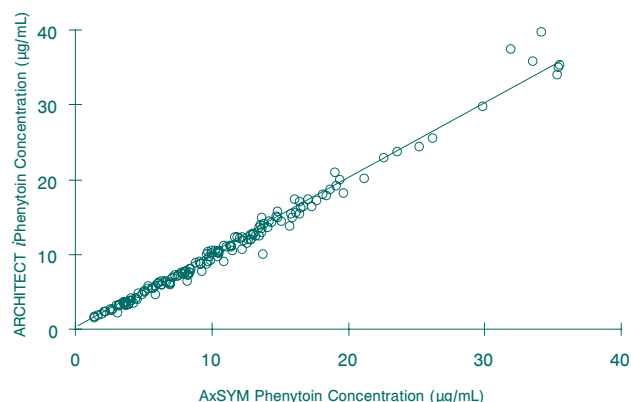
Specimen Range (ARCHITECT) = 1.45 µg/mL to 39.63 µg/mL

Specimen Range (AxSYM) = 1.44 µg/mL to 35.49 µg/mL

^a A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.²³

^b Confidence Interval (CI)

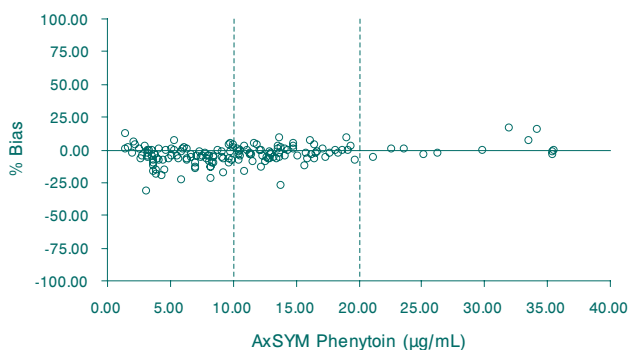
ARCHITECT *i*Phenytoin vs AxSYM Phenytoin



* Representative data; results in individual laboratories may vary from these data.

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 35.49 µg/mL, respectively. The following representative data are provided to aid in understanding the difference between the two assays. 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, as read in the AxSYM), the average bias was -2.73% with a 95% confidence interval of -14.61 to 9.15%. Results of the study are summarized below.* The vertical lines depict the typical therapeutic range of phenytoin therapy.

ARCHITECT i Phenytoin % Bias to AxSYM Phenytoin



* Representative data; results in individual laboratories may vary from these data.

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Related Reading


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