ARCHITECT SYSTEM

en

*i*Phenobarbital

REF 1P33

F5-O204-2/R2

# *i*Phenobarbital

**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.

**Read Highlighted Changes**
**Revised October, 2008**

## Key to symbols used

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

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

## NAME

ARCHITECT *i* Phenobarbital

## INTENDED USE

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.

## SUMMARY AND EXPLANATION OF TEST

Phenobarbital was introduced in 1912 for the treatment of epilepsy, particularly for controlling focal motor or sensory seizures and grand mal seizures.[1] Phenobarbital is bound to both plasma and tissue proteins.[2] Monitoring serum concentrations of phenobarbital has been shown to improve patient therapy by providing physicians with a tool for adjusting dosage.[3] In addition, because of the narrow therapeutic index and wide inter-individual variability in the rate of phenobarbital metabolism and clearance, the determination of blood levels of phenobarbital for patients receiving therapy is essential.[4]

## BIOLOGICAL PRINCIPLES OF THE PROCEDURE

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

Sample, anti-phenobarbital coated paramagnetic microparticles, and phenobarbital acridinium-labeled conjugate are combined to create a reaction mixture. The anti-phenobarbital 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.

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

## REAGENTS

### Reagent Kit, 100 Tests

ARCHITECT *i* Phenobarbital Reagent Kit (1P33)

- **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.
- **CONJUGATE** 1 Bottle (5.9 mL) Phenobarbital acridinium-labeled conjugate in MES buffer with surfactant. Minimum concentration: 1.2 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

- **IVD** **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 2[6] or other appropriate biosafety practices[7,8] should be used for materials that contain or are suspected of containing infectious agents.
- All 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* Phenobarbital 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* Phenobarbital 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* Phenobarbital 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* Phenobarbital 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* Phenobarbital 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 (4.31) = 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* Phenobarbital 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 heparin
  - sodium EDTA
  - potassium oxalate
- 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* Phenobarbital assay.

### Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated specimens
  - grossly hemolyzed
  - 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.[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 six months at -20°C or colder.[9]

### 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

- 1P33 ARCHITECT *i* Phenobarbital 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
- 1P33-01 ARCHITECT *i* Phenobarbital 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* Phenobarbital 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* Phenobarbital 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* Phenobarbital test plus 20 µL for each additional ARCHITECT *i* Phenobarbital test from the same sample cup.
  - ≤ 3 hours on board: 150 µL for the first ARCHITECT *i* Phenobarbital test plus 20 µL for each additional ARCHITECT *i* Phenobarbital 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* Phenobarbital Calibrators and controls should be prepared according to their respective package inserts.
  - To obtain the recommended volume requirements for the ARCHITECT *i* Phenobarbital 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 phenobarbital value exceeding 80.00 µg/mL are flagged with the code ">80.00" and may be diluted with the Manual Dilution Procedure.

- Manual dilutions should be performed as follows:
  - The suggested dilution for a phenobarbital test is 1:10.
  - Add 10 µL of the patient specimen to 90 µL of ARCHITECT *i* Phenobarbital 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.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

### Calibration

- To perform an ARCHITECT *i* Phenobarbital calibration, test calibrators A, B, C, D, E, and F in duplicate. A single sample of each phenobarbital 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 - 80.0 µg/mL.
- Once an ARCHITECT *i* Phenobarbital 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* Phenobarbital 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.

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* Phenobarbital assay belongs to method group 2.

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

## RESULTS

### Calculation

The ARCHITECT *i* Phenobarbital 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* Phenobarbital assay is 1.10 µg/mL to 80.00 µg/mL.

## LIMITATIONS OF THE PROCEDURE

- If the ARCHITECT *i* Phenobarbital 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.
- Amobarbital and mephobarbital are drugs structurally similar to phenobarbital. These drugs may interfere with the ARCHITECT *i* Phenobarbital assay.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).[10,11] Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies.[11]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[12] 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

**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.

Strong correlations have been shown between serum levels of phenobarbital and both therapeutic effect and toxicity.[13] Clinical observations indicate that toxicity of phenobarbital is increased in patients with renal disease.[14] 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.[15] Some patients under chronic treatment develop macrocytosis and megaloblastic anemia as well as osteomalacia.[16-18] Most patients will receive maximum seizure control when serum levels of phenobarbital are in the range of 15-40 µg/mL.[19]

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

## SPECIFIC PERFORMANCE CHARACTERISTICS

### Precision

The ARCHITECT *i* Phenobarbital assay is designed to have an assay precision of ≤ 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 table.*

| | Instru- | Reagent | | Mean | Within Run | | Total | |
|---|---|---|---|---|---|---|---|---|
| Sample | ment | Lot | n | (µg/mL) | SD | %CV | SD | %CV |
| Level 1 | 1 | 1 | 80 | 9.37 | 0.31 | 3.31 | 0.34 | 3.63 |
| | 2 | 2 | 80 | 9.26 | 0.32 | 3.46 | 0.36 | 3.89 |
| | 3 | 3 | 80 | 9.40 | 0.24 | 2.55 | 0.33 | 3.51 |
| Level 2 | 1 | 1 | 80 | 23.59 | 0.72 | 3.05 | 0.82 | 3.48 |
| | 2 | 2 | 80 | 23.34 | 0.73 | 3.13 | 1.01 | 4.33 |
| | 3 | 3 | 80 | 24.11 | 0.63 | 2.61 | 0.70 | 2.90 |
| Level 3 | 1 | 1 | 80 | 47.30 | 1.21 | 2.56 | 1.52 | 3.21 |
| | 2 | 2 | 80 | 48.51 | 1.38 | 2.84 | 1.48 | 3.05 |
| | 3 | 3 | 80 | 48.87 | 1.28 | 2.62 | 1.45 | 2.97 |
| Panel 1 | 1 | 1 | 80 | 9.64 | 0.32 | 3.32 | 0.33 | 3.42 |
| | 2 | 2 | 80 | 9.46 | 0.27 | 2.85 | 0.35 | 3.70 |
| | 3 | 3 | 80 | 9.68 | 0.27 | 2.79 | 0.32 | 3.31 |
| Panel 2 | 1 | 1 | 80 | 37.15 | 0.97 | 2.61 | 1.22 | 3.28 |
| | 2 | 2 | 80 | 37.36 | 1.06 | 2.84 | 1.41 | 3.77 |
| | 3 | 3 | 80 | 38.75 | 1.13 | 2.92 | 1.28 | 3.30 |
| Panel 3 | 1 | 1 | 80 | 56.21 | 1.52 | 2.70 | 2.09 | 3.72 |
| | 2 | 2 | 80 | 57.76 | 2.44 | 4.22 | 2.65 | 4.59 |
| | 3 | 3 | 80 | 57.32 | 1.58 | 2.76 | 2.00 | 3.49 |

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

In order to assess the precision near the high end of the dynamic range (80.00 µg/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 | Instrument | Reagent Lot | n | Mean (µg/mL) | Within Run SD | Within Run %CV | Total SD | Total %CV |
|---|---|---|---|---|---|---|---|---|
| High Panel | 1 | 1 | 20 | 76.98 | 1.40 | 1.82 | 1.93 | 2.51 |
| | 2 | 2 | 20 | 71.18 | 2.18 | 3.06 | 2.93 | 4.11 |
| | 3 | 3 | 20 | 75.07 | 3.03 | 4.04 | 3.09 | 4.12 |

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

**Recovery**

The ARCHITECT *i* Phenobarbital assay is designed to have a mean recovery of 100 ± 10%.

A study was performed on five serum samples and five plasma samples, where phenobarbital was spiked into the samples to target concentrations of 0, 8, 16, 40, and 60 µg/mL. The concentration of phenobarbital was determined using the ARCHITECT *i* Phenobarbital assay and the resulting percent recovery was calculated. The mean percent recovery of the ARCHITECT *i* Phenobarbital assay for serum ranged from 94% to 99% and for plasma ranged from 95% to 100% with a grand mean for serum and plasma of 97%.*

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

**Linearity**

The ARCHITECT *i* Phenobarbital assay is designed to have a mean recovery of 100 ± 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* Phenobarbital Calibrator A. The concentration of phenobarbital was determined using the ARCHITECT *i* Phenobarbital assay and the resulting percent recovery was calculated. Data from this study are summarized in the following tables.*

**ARCHITECT *i* Phenobarbital Serum Samples**

| Specimen | Dilution Factor | Observed Concentration (µg/mL) | % Recovery[a] |
|---|---|---|---|
| 1 | 1 | 76.88 | - |
| | 4 | 19.69 | 102.4 |
| | 10 | 8.32 | 108.2 |
| | 40 | 2.28 | 118.8 |
| 2 | 1 | 77.82 | - |
| | 4 | 19.31 | 99.2 |
| | 10 | 7.98 | 102.5 |
| | 40 | 2.13 | 109.5 |
| 3 | 1 | 77.83 | - |
| | 4 | 20.30 | 104.3 |
| | 10 | 8.03 | 103.2 |
| | 40 | 2.14 | 109.8 |
| | | | Mean = 106.4% |

**ARCHITECT *i* Phenobarbital Plasma Samples**

| Specimen | Dilution Factor | Observed Concentration (µg/mL) | % Recovery |
|---|---|---|---|
| 1 | 1 | 76.09 | - |
| | 4 | 18.65 | 98.0 |
| | 10 | 7.69 | 101.1 |
| | 40 | 2.00 | 105.1 |
| 2 | 1 | 78.36 | - |
| | 4 | 19.31 | 98.6 |
| | 10 | 8.07 | 103.0 |
| | 40 | 2.17 | 111.0 |
| 3 | 1 | 73.68 | - |
| | 4 | 20.16 | 109.4 |
| | 10 | 7.82 | 106.1 |
| | 40 | 2.18 | 118.2 |
| | | | Mean = 105.6% |

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

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

**Sensitivity**

Analytical Sensitivity

Analytical sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus two times the SD obtained on the blank sample. The ARCHITECT *i* Phenobarbital assay is designed to have a sensitivity of ≤ 1.10 µg/mL.

Limit of Blank (LoB) and Limit of Detection (LoD)

The LoB and LoD of the ARCHITECT *i* Phenobarbital assay were determined with guidance from CLSI Protocol EP17-A[21] 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 phenobarbital samples (15 replicates each); LoB = 0.15 µg/mL and LoD = 0.34 µg/mL.*

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

**Specificity**

The specificity of the ARCHITECT *i* Phenobarbital assay was determined by studying the cross-reactivity of compounds whose chemical structure or concurrent usage could cause potential interference with the ARCHITECT *i* Phenobarbital assay. Specificity of the assay was determined by spiking each compound into human serum specimens with phenobarbital levels targeted at 15 and 40 µg/mL. The average amount of interference observed during the study ranged from -0.2% to 5.5%* except for amobarbital and mephobarbital. The average amount of interference observed during the study for amobarbital was 21.9%* and for mephobarbital > 100.0%*.

| Test Compound | Concentration (µg/mL) |
|---|---|
| Amitriptyline | 25 |
| Amobarbital[a] | 30 |
| Aprobarbital | 100 |
| Barbital | 100 |
| Butabarbital | 100 |
| Carbamazepine-10,11-epoxide | 240 |
| Chlordiazepoxide | 100 |
| Chlorpromazine | 100 |
| Chlorazepate | 100 |
| Ethotoin | 300 |
| 5-Ethyl-5-phenylhydantoin | 200 |
| p-Hydroxyphenobarbital | 22 |
| Imipramine | 20 |
| Mephobarbital[a] | 15 |
| Methsuximide | 150 |
| Pentobarbital | 100 |
| Phenytoin | 300 |
| Primidone | 200 |
| Secobarbital | 25 |
| Thiopental | 100 |

a Refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert.

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

### Interference

Potential interference in the ARCHITECT *i* Phenobarbital 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-A2[22] was performed for the ARCHITECT *i* Phenobarbital assay. Serum specimens with phenobarbital levels were targeted at 15 and 40 µg/mL and supplemented with the following potentially interfering compounds. The mean recovery in this study ranged from 92.1% to 101.2%.*

| Potentially Interfering Compound | Concentration |
|---|---|
| Triglycerides | 2500 mg/dL |
| Hemoglobin | 500 mg/dL |
| Bilirubin | 15 mg/dL |
| Low Protein | 3 g/dL |
| High Protein | 10 g/dL |

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

Evaluation of Other Potentially Interfering Compounds

The ARCHITECT *i* Phenobarbital 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* Phenobarbital 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 percent recovery with phenobarbital spiked into each specimen to target concentrations of 15 and 40 µg/mL. The mean percent recovery for HAMA specimens ranged from 97.0% to 100.1% and for RF specimens ranged from 99.3% to 99.6%.*

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

### Method Comparison

The ARCHITECT *i* Phenobarbital assay is designed to have a slope of 1.00 ± 0.15 and a correlation coefficient (r) of ≥ 0.95 for specimens when compared to AxSYM Phenobarbital. A study was performed using serum specimens. The data were analyzed using the Passing-Bablok[a] regression method and are summarized in the following table.*

**ARCHITECT *i* Phenobarbital vs. AxSYM Phenobarbital**

| Number of Observations | Slope (95% CI[b]) | Intercept (95% CI) | Correlation Coefficient |
|---|---|---|---|
| 132 | 0.93 (0.91 to 0.95) | -0.44 (-0.80 to -0.18) | 1.0 |

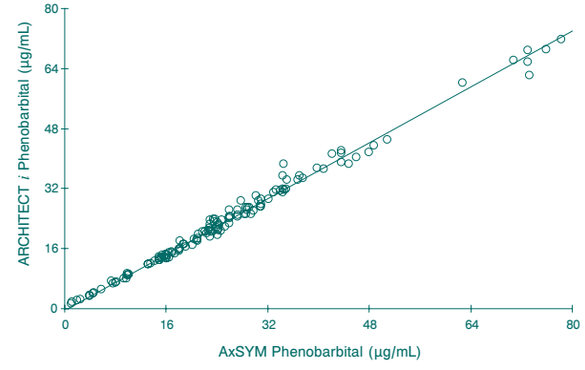
Specimen Range (ARCHITECT) = 1.42 µg/mL to 71.65 µg/mL

Specimen Range (AxSYM) = 1.10 µg/mL to 78.25 µg/mL

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

b Confidence Interval

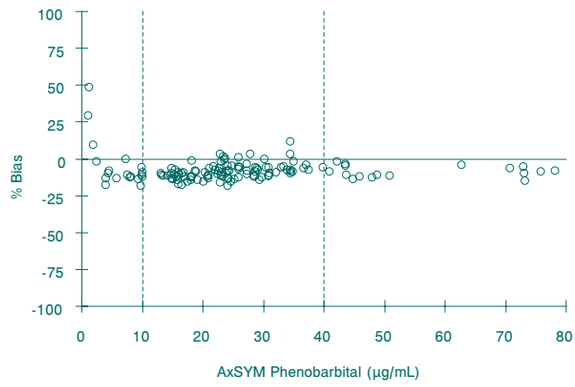
ARCHITECT *i* Phenobarbital vs. AxSYM Phenobarbital




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

A bias analysis of ARCHITECT *i* Phenobarbital 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 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 -8.1%. The 95% confidence interval of that average bias is -23.9% to 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% with a 95% confidence interval of -18.9% to 1.5%. Results of the study are summarized in the following graph.* The vertical lines depict the typical therapeutic range of phenobarbital therapy.

ARCHITECT *i* Phenobarbital % Bias to AxSYM Phenobarbital




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

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Related Reading

Physicians' Desk Reference, 58th Edition, Montvale, NJ: Medical Economics Co Inc., 2004.



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