SIEMENS

# Syva®

# *Emit® 2000* Phenytoin Assay

See shaded sections: Updated information from 2015-03 version.

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## Phenytoin Assay

### 1 Intended Use

The Emit® 2000 Phenytoin Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of phenytoin in human serum or plasma. Emit® 2000 assays are designed for use with most chemistry analyzers.

### 2 Summary and Explanation of the Test

Monitoring serum phenytoin concentrations, along with careful clinical assessment, is the most effective means of improving seizure control, reducing the risk of toxicity, and minimizing the need for additional anticonvulsant medication for the following reasons:[1,2]

- Serum phenytoin concentrations correlate better with pharmacologic activity than does dosage because of individual differences in absorption, metabolism, disease states, concomitant medication, and compliance. Serum concentration monitoring helps physicians individualize dosage regimens.
- The hepatic enzyme system for metabolizing phenytoin can become saturated within the drug's therapeutic range. When this occurs, small dosage alterations can lead to unexpected drug accumulation and clinical toxicity.
- Phenytoin is safe and effective only in a narrow range of serum concentrations.

Methods historically used to monitor serum phenytoin concentrations include chromatographic assays and immunoassays.[1–4]

### 3 Principle

The Emit® 2000 assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.[5] The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

### 4 Reagents

| REF | Product Description | Quantity/Volume |
|---|---|---|
| 4A019UL | **Emit® 2000 Phenytoin Assay Antibody/Substrate Reagent 1** mouse monoclonal antibodies reactive to phenytoin (53.4 µg/mL),* glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), preservatives, including 0.1% sodium azide, and stabilizers | 28 mL |
| | **Enzyme Reagent 2** phenytoin labeled with bacterial glucose-6-phosphate dehydrogenase (0.24 U/mL),* Tris buffer, preservatives, including 0.1% sodium azide, and stabilizers | 14 mL |

**** The antibody titer and enzyme conjugate activity may vary from lot to lot.***

***Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.***

For *in vitro* diagnostic use.

**Precautions**

- Contains sodium azide as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.
- This kit contains streptomycin sulfate. Please dispose of appropriately.

**Preparation and Storage of Assay Components**

**Emit® 2000 Phenytoin Assay**

The Emit® 2000 Phenytoin Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent vials when not in use. Always return the reagent screw caps to their original vials.

Store reagents at 2–8°C (36–46°F), upright, and with screw caps tightly closed when not in use. Reagents are stable until the expiration date printed on the label if stored as directed. Do not freeze reagents or expose them to temperatures above 32°C. **Improper storage of reagents can affect assay performance.**

### 5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, citrate, oxalate, and EDTA have been tested and may be used with this assay. Some sample dilution may occur when samples are collected in tubes containing citrate anticoagulant. The amount of dilution and the possible need to correct for it should be considered when interpreting assay results for these samples.
- Sample volume is instrument-dependent. Refer to the appropriate instrument application instructions for specific volumes.
- Store the serum or plasma refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C. Samples can be stored refrigerated at 2–8°C for up to one month or stored frozen for up to three months.[6]
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.[1,2] Draw a sample within 2 to 4 hours after an intravenous loading dose and, at steady state, collect a specimen representing the trough level just before the next scheduled dose.[1]
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.

### 6 Procedure

**Materials Provided**

Emit® 2000 Phenytoin Assay
Reagent 1
Reagent 2

**Materials Required But Not Provided**

4A109UL Emit® 2000 Phenytoin Calibrators (0, 2.5, 5, 10, 20, 40 µg/mL)
Multi-level commercial controls

**Instruments**
Siemens Healthcare Diagnostics provides instructions for using this assay on a number of chemistry analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for application sheets.

Analyzers must be capable of maintaining a constant reaction temperature, pipetting specimens/reagents and measuring enzyme rates precisely, timing the reaction accurately, and mixing reagents thoroughly.

**Calibration**

Recalibrate whenever a new lot of reagents is used or as indicated by control results (see Quality Control, below). If a new set of reagents with the same lot number is used, validate the system by assaying controls.

**Quality Control**

1. Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.
2. Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known phenytoin concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
3. Refer to the instrument operator's manual for appropriate instrument checks.

**Diluting High Concentration Samples**

To estimate phenytoin concentrations above the assay range, patient samples containing more than 40 µg/mL (158 µmol/L) phenytoin may be diluted with either one or two parts distilled or deionized water or Emit® 2000 Phenytoin Calibrator 0. After diluting the sample, repeat the entire assay sequence and multiply the results by-the dilution factor. Some analyzers dilute and retest high concentration samples automatically. See the appropriate protocol sheet for instructions.

**Daily Maintenance**

Refer to the instrument operator's manual for maintenance instructions.

## 7 Results

- Results are calculated automatically by the analyzers. No additional manipulation of data is required.
- This assay uses Math Model No. 1.
- Consult the appropriate instrument operator's manual and protocol sheet for complete instructions.
- The factors that can influence the relationship between phenytoin serum or plasma concentrations and clinical response include the type and severity of seizures, age, general state of health, and use of other drugs.
- The concentration of phenytoin in serum or plasma depends on the time of the last drug dose; mode of administration; concomitant drug therapy; sample condition; time of sample collection; and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.[1,2]
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.
- Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.

## 8 Limitations

This assay has no specific limitations.

## 9 Expected Values

The Emit® 2000 Phenytoin Assay accurately quantitates phenytoin concentrations in human serum or-plasma containing 2.5–40 µg/mL (10–158 µmol/L) phenytoin. Most patients achieve a satisfactory therapeutic response in the serum concentration range of 10–20 µg/mL (40–79 µmol/L).[1,2,5,7] Further, peak concentrations above 20 µg/mL (79 µmol/L) are often associated with toxicity.[1,5,7]

***Note: To convert from µg/mL to µmol/L phenytoin, multiply by 3.96.***

For effective treatment, some patients may require serum levels outside these ranges. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms (see Section 7, Results).

## 10 Specific Performance Characteristics

The data appearing in this section were collected on the Hitachi 704 Analyzer and the COBAS MIRA® Chemistry System. Performance characteristics (including calibration stability) are available for a variety of analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for information for specific analyzers.

The following performance characteristics information represents total system performance and should not be interpreted to pertain only to reagents. Performance may vary depending on the instrumentation used.

**Specificity**

The Emit® 2000 Phenytoin Assay measures the total (protein-bound plus unbound) phenytoin concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested.

The compounds listed in Table 1 do not interfere with the Emit® 2000 Phenytoin Assay when tested in the presence of 10 µg/mL phenytoin. Levels tested were at or above maximum physiological or pharmacological concentrations.

**Table 1 — Compounds That Do Not Interfere**

| Compound | Concentration Tested (µg/mL) | Compound | Concentration Tested (µg/mL) |
|---|---|---|---|
| Amitriptyline | 25 | Imipramine | 5 |
| Amobarbital | 75 | Mephenytoin | 35 |
| Carbamazepine | 500 | Mephobarbital | 500 |
| Carbamazepine-10,11-epoxide | 500 | Methsuximide | 150 |
| Chlordiazepoxide | 60 | Pentobarbital | 100 |
| Chlorpromazine | 8 | Phenobarbital | 500 |
| Clorazepate | 500 | Phensuximide | 500 |
| Diazepam | 60 | 2-Phenyl-2-ethyl-malondiamide (PEMA) | 500 |
| Ethosuximide | 500 | Primidone | 200 |
| Ethotoin | 200 | Promethazine | 10 |
| 5-Ethyl-5-phenylhydantoin | 200 | Secobarbital | 25 |
| Glutethimide | 200 | Valproic Acid | 1000 |
| 5-(p-Hydroxyphenyl)-5-phenylhydantoin | 50 | | |
| 5-(p-Hydroxyphenyl)-5-phenylhydantoin glucuronide | 1000 | | |

For additional information, contact Siemens.

**Sensitivity**

The sensitivity level of the Emit® 2000 Phenytoin Assay is 0.5 µg/mL. This level represents the lowest measurable concentration of phenytoin that can be distinguished from 0 µg/mL with a confidence level of 95%.

**Endogenous Substances**

No clinically significant interference has been found in samples to which 800 mg/dL hemoglobin, 750 mg/dL triglycerides, and 30 mg/dL bilirubin were added to simulate hemolytic, lipemic, or icteric samples.

**Calibration Stability**

In-house and field studies have shown calibration stability of more than two weeks. When proper reagent handling, instrument maintenance, and operating procedures are followed, the calibration should remain stable for at least two weeks.

**Precision**

The precision values shown in Tables 2 and 3 were obtained in clinical trials using the Hitachi 704 and COBAS MIRA® analyzers.

Within-run precision was determined using tri-level commercial controls.

**Table 2 — Within-Run Precision for Emit® 2000 Phenytoin Assay on the Hitachi 704 and COBAS MIRA® Analyzers**

| Site/ Analyzer | Number of Replicates | Mean (µg/mL) | Coefficient of Variation (%) |
|---|---|---|---|
| **Site 1** | 20 | 4.1 | 4.6 |
| Hitachi 704 | 20 | 12.3 | 4.8 |
| | 20 | 24.2 | 7.3 |
| **Site 2** | 20 | 3.9 | 5 |
| COBAS MIRA® | 20 | 12.2 | 5.4 |
| | 20 | 23.6 | 7 |
| **Site 3** | 20 | 4.4 | 8.6 |
| COBAS MIRA® | 20 | 11.7 | 8.7 |
| | 20 | 22.5 | 10.2 |

Between-run precision was determined using tri-level commercial controls

**Table 3 — Between-Run Precision for Emit® 2000 Phenytoin Assay on the Hitachi 704 and COBAS MIRA® Analyzers**

| Site/ Analyzer | Length of Study (days) | Number of Replicates | Mean (µg/mL) | Coefficient of Variation (%) |
|---|---|---|---|---|
| **Site 1** | 85 | 60 | 3.8 | 8.5 |
| Hitachi 704 | | 60 | 12.4 | 9.5 |
| | | 60 | 24.4 | 8.7 |
| **Site 2** | 26 | 60 | 4.4 | 6.4 |
| COBAS MIRA® | | 60 | 13.3 | 6.8 |
| | | 60 | 25.2 | 8.2 |
| **Site 3** | 15 | 60 | 3.8 | 8.2 |
| COBAS MIRA® | | 60 | 11.6 | 7.7 |
| | | 60 | 21.9 | 10.3 |

**Accuracy**

Samples from patients receiving phenytoin were analyzed by the Emit® 2000 Phenytoin Assay on the Hitachi 704 or COBAS MIRA® analyzers, and the results were compared to two alternative methods: Emit® Phenytoin Assay on the COBAS MIRA® analyzer and fluorescence polarization immunoassay (FPIA). Data are summarized in Table 4.

**Table 4 — Comparative Analysis**

| | Emit® 2000* vs FPIA | Emit® 2000† vs FPIA | Emit® 2000† vs Emit®† |
|---|---|---|---|
| Slope | 1.00 | 0.95 | 0.94 |
| Intercept (µg/mL) | -1.92 | -1.84 | -0.69 |
| Mean (µg/mL) | | | |
| Emit® 2000 Assay | 19.3 | 14.9 | 15.3 |
| Comparison Method | 21.2 | 17.6 | 16.9 |
| Standard Error of the Estimate (µg/mL) | 1.47 | 1.43 | 0.97 |
| Correlation Coefficient | 0.98 | 0.96 | 0.98 |
| Number of Samples | 97 | 105 | 83 |

**** This assay was performed on the Hitachi 704 analyzer.***

***† This assay was performed on the COBAS MIRA® analyzer.***

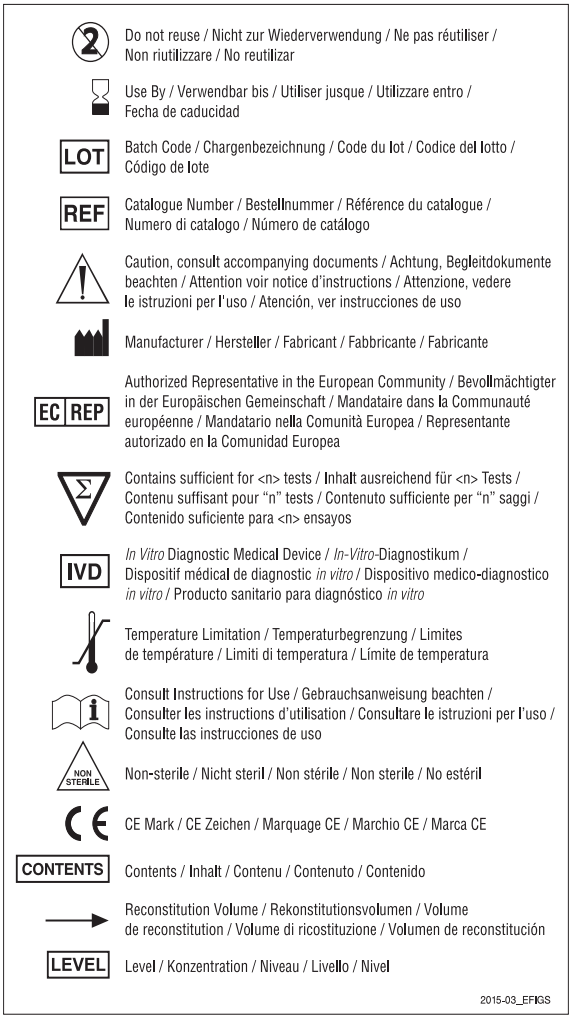
## 11 Risk and Safety

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

## 12 Bibliography


1. Finn AL, Olanow CW: Phenytoin: Therapeutic use and serum concentration monitoring, in Taylor WJ, Finn AL (eds): *Individualizing Drug Therapy: Practical Applications of Drug Monitoring*. New York, Gross, Townsend, Frank, Inc, 1981, vol 2, pp 63–85.
2. Winter ME, Tozer TN: Phenytoin, in Evans WE, Schentag JJ, Jusko WJ (eds): *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring,* ed 2. Washington, Applied Therapeutics, Inc, 1987, pp 493–539.
3. Leppik IE, Oles KS, Sheehan ML, et al: Phenytoin and phenobarbital concentrations in serum: A comparison of Ames Seralyzer with GLC, TDX, and Emit. *Ther Drug Monit* 1989;11:73–78.
4. Rainbow SJ, Dawson CM, Tickner TR: Direct serum injection high-performance liquid chromatographic method for the simultaneous determination of phenobarbital, carbamazepine, and phenytoin. *J Chromatogr* 1990;527:389–396.
5. Pincus MR, Abraham NZ Jr: Toxicology and therapeutic drug monitoring, in Henry JB (ed): *Clinical Diagnosis and Management by Laboratory Methods,* ed 18. Philadelphia, WB Saunders Co, 1991, pp 349–384.
6. Kumps AH: Therapeutic drug monitoring: A comprehensive and critical review of analytical methods for anticonvulsive drugs. *J Neurol* 1982;228:1–16.
7. Levine M, McCollom R, Chang T, et al: Evaluation of serum phenytoin monitoring in an acute care setting. *Ther Drug Monit* 1988;10:50–57.


## 13 Symbols Key




**For technical assistance, call Siemens Healthcare Diagnostics:**
**1-800-227-8994 in the USA**
**1-800-264-0083 in Canada**
**Outside the USA and Canada, call your local Siemens representative.**

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