BECKMAN COULTER

Emit® 2000 Phenytoin Assay

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See shaded sections:
Updated information from 2017-03 edition.

Catalog Number	Product Description	Quantity/ Volume
OSR4A229	**Emit® 2000 Phenytoin Assay**	
	OSR4A518 R1 (Antibody/Substrate Reagent 1)	2 x 21 mL
	OSR4A548 R2 (Enzyme Reagent 2)	2 x 16 mL
4A109UL	**Emit® 2000 Phenytoin Calibrators***	1 x 5 mL†, 5 x 2 mL

Required for calibrating the Emit ® 2000 Phenytoin Assay. Sold separately.

†Additional negative calibrator is provided.

Note: Reagents and calibrators are shipped ready to use in liquid form.

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

The Emit® 2000 Phenytoin Calibrators contain the following stated phenytoin concentrations:

Calibrator	0	2.5	5	10	20	40
Phenytoin (µg/mL	0	2.5	5.0	10	20	40
Phenytoin (µmol/L)	0	10	20	40	79	158

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. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

2 SUMMARY

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 anti-convulsant 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 METHODOLOGY

The Emit® 2000 Phenytoin Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.[5,6] 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

Reagents contain the following substances:

Mouse monoclonal antibodies reactive to phenytoin (53.4 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), phenytoin labeled with glucose-6-phosphate dehydrogenase (0.24 U/mL), Tris buffer, 0.1% sodium azide, preservatives, and stabilizers.

Risk and Safety

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

Precautions

- For *in vitro* diagnostic use.
- Contains nonsterile mouse monoclonal antibodies.
- Assay components contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- Do not use the kit after the expiration date.
- This kit contains streptomycin sulfate. Please dispose of appropriately.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents

The Emit® 2000 Phenytoin Assay reagents are provided ready to use; no preparation is necessary.

Storage of Assay Components

- Improper storage of reagents can affect assay performance.
- When not in use, store reagents upright at 2–8°C and with screw caps tightly closed.
- Unopened reagents are stable until the expiration date printed on the label if stored upright at 2–8°C.
- Do not freeze reagents or expose them to temperatures above 32°C.

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 Application Sheet 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.[7]
- 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 two to four 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

Emit® 2000 Phenytoin Calibrators
Multi-level commercial controls

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

- 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.
- 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.
- Refer to the instrument User's Guide for appropriate instrument checks and maintenance instructions.

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 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 analyzer User's Guide or appropriate Application Sheet for instructions.

Evaluation and Interpretation of Results

- This assay uses Math Model No. 1.
- Results are automatically calculated; no additional manipulation of data is required.
- 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.

7 LIMITATIONS OF THE PROCEDURE

This assay has no specific limitations.

8 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).[1,2,5,8] Further, peak concentrations above 20 µg/mL (79 µmol/L) are often associated with toxicity.[1,5,8]

For patients being treated with fosphenytoin (Cerebyx®), it is important not to collect samples for phenytoin analysis until at least 2 hours after the completion of intravenous infusion, or 4 hours after intramuscular injection, when conversion of the prodrug to phenytoin can be expected to be essentially complete.[9–11]

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.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 Phenytoin Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Endogenous Substances

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

Precision

Within-run precision was determined by assaying 20 replicates of each level of a tri-level control. Table 1 summarizes the data.

Table 1 — Summary of Within-Run Precision

	Level 1	Level 2	Level 3
Mean (µg/mL)	4.23	12.87	22.51
%CV	3.8	3.1	3.3

Total precision was calculated according to NCCLS guideline EP5-T2 using data collected from controls run in duplicate twice daily over twenty (20) days. Table 2 summarizes the data.

Table 2 — Summary of Total Precision

	Level 1	Level 2	Level 3
Mean (µg/mL)	4.03	11.71	23.12
%CV	6.0	5.9	8.2

Comparative Analysis

Samples from patients were analyzed on the Roche Diagnostics(RD)/Hitachi 704 and the AU600, and the results were compared. Table 3 presents these results.

Table 3 — Summary of Comparative Analysis

Slope		1.06
Intercept		-0.52
Mean	RD/Hitachi 704	15.37
	AU600	15.75
Correlation Coefficient		0.97
Number		55

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 4 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 4 — Compounds That Do Not Interfere

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

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%.

Calibration Stability

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.

10 REFERENCES

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Symbols Key

	Do not reuse
	Use By
LOT	Batch Code
REF	Catalogue Number
	Caution, consult accompanying documents
	Manufacturer
EC REP	Authorized Representative in the European Community
	Contains sufficient for <n> tests
IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation
	Consult Instructions for Use
NON STERILE	Non-sterile
CE	CE Mark
CONTENTS	Contents
	Reconstitution Volume
LEVEL	Level

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