Emit® II Plus Barbiturate Assay

September 2013 9D052.5D_D

See shaded sections:
Updated information from October 2011 version.

Catalog Number	Product Description	Quantity/ Volume
OSR9D229	**Emit® II Plus Barbiturate Assay**	
	OSR9D618 R1 (Antibody/Substrate Reagent 1)	2 x 31 mL
	OSR9D648 R2 (Enzyme Reagent 2)	2 x 15 mL
9A509UL	**Emit® Calibrator/Control Level 0***	1 x 14 mL
9A549UL	**Emit® Calibrator/Control Level 2 (100)***	1 x 14 mL
9A569UL	**Emit® Calibrator/Control Level 3 (200)***	1 x 14 mL
9A589UL	**Emit® Calibrator/Control Level 4 (300)***	1 x 14 mL
9A609UL	**Emit® Calibrator/Control Level 5 (800)***	1 x 14 mL

**** Required for calibrating the Emit® II Plus Barbiturate Assay. Sold separately. To determine the appropriate calibrators required for use, see Table 1.***

Note: Reagents and calibrators/controls are shipped ready to use in liquid form. No reconstitution is required.

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

Note: These reagents are qualified for use with these calibrators only. However, other material may be used for quality control purposes.

Table 1 — Emit® Calibrators/Controls for Use in Qualitative or Semiquantitative Analysis

	Qualitative Analysis		Semiquantitative Analysis	
Desired Cutoff Level (ng/mL)	Required Cal/Control Level	Concentration of Secobarbital (ng/mL)	Required Cal/Control Level	Concentration of Secobarbital (ng/mL)
200	Level 0 Level 3 Level 5	0 200 800	Level 0 Level 2 Level 3	0 100 200
300	Level 0 Level 4 Level 5	0 300 800	Level 4 Level 5	300 800

Note: The Emit® Calibrators/Controls contain the stated concentrations of secobarbital listed in Table 1. Emit® Calibrator/Control Levels 2, 3, 4, and 5 contain additional drugs of abuse that do not affect the assay. For any individual cutoff listed, a calibrator/control is used either as a calibrator or as a control when the assay is used for qualitative analysis. When a calibrator/control is used as a calibrator for an individual cutoff level, the other level calibrators/controls (above or below it, as listed above) are used as controls. See the Emit® Calibrators/Controls instructions for use.

1 INTENDED USE

The Emit® II Plus Barbiturate Assay is a homogeneous enzyme immunoassay with a 200 ng/mL or 300 ng/mL cutoff. The assay is intended for use in the qualitative and semiquantitative analyses of barbiturates in human urine. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

The Emit® II Plus Barbiturate Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when preliminary positive results are used.

2 SUMMARY

Barbiturates, a class of nervous system depressants, are usually taken orally, but are sometimes injected intravenously or intramuscularly. They are absorbed rapidly; 30–40% is bound to plasma protein, and the rest is distributed to muscle, fat, and to the liver (where they are ultimately inactivated).[2] They are classified based on their duration of action, ranging from very short acting (approximately 15 minutes) to long acting (a day or more). Some of the most commonly abused barbiturates are the short-acting ones, including pentobarbital and secobarbital. An example of a long-acting barbiturate is phenobarbital. The ratio of unchanged drug to metabolites varies depending upon duration of action. Short-acting barbiturates will generally be excreted in urine as metabolites, while the long-acting barbiturates will primarily appear unchanged.[3,4]

The Emit® II Plus Barbiturate Assay, an enzyme immunoassay technique, tests for both long- and short-acting barbiturates in human urine. Positive results for specimens containing other compounds structurally unrelated to barbiturates have not been observed. The cutoff levels for distinguishing positive from negative specimens are 200 ng/mL and 300 ng/mL.

Methods historically used for detecting barbiturates in biological fluids include thin-layer chromatography, gas chromatography, ultraviolet spectrophotometry, enzyme immunoassay, and radioimmunoassay.[5]

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.[1]

3 METHODOLOGY

The Emit® II Plus Barbiturate Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[6] The assay is based on competition between drug in the specimen 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 specimen can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme NAD functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 REAGENTS

Reagents contain the following substances:

Sheep polyclonal antibodies to secobarbital (3.2 µg/mL), glucose-6-phosphate (15 mM), nicotinamide adenine dinucleotide (12 mM), bovine serum albumin, secobarbital labeled with G6PDH (0.47 U/mL), Tris buffer, preservatives, and stabilizers.

Precautions

- For *in vitro* diagnostic use.
- Reagent 1 contains nonsterile sheep antibodies.
- Reagent 2 contains nonsterile mouse antibodies.
- Reagents 1 and 2 contain nonsterile bovine serum albumin.
- Do not use after expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

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

Preparation of Reagents

The Emit® II Plus Barbiturate 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

- Urine specimens may be collected in plastic (ie, polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics can adsorb certain drugs.
- Internal testing has shown that if not analyzed immediately, specimens may be stored unrefrigerated for up to 7 days. Specimens may be stored refrigerated for 30 days before analysis. After 7 days unrefrigerated or 30 days refrigerated, samples should be stored frozen.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Specimens with high turbidity should be centrifuged before analysis.
- The recommended pH range for urine specimens is 3.0–11.0.
- Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.
- Human urine specimens should be handled and treated as if they were potentially infectious.

6 PROCEDURE

Materials Provided

Emit® II Plus Barbiturate Assay
- Reagent 1
- Reagent 2

Materials Required But Not Provided

Emit® Calibrators/Controls
Commercially available controls (see Quality Control, Semiquantitative Analysis)

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

Calibration

Qualitative Analysis

Run the appropriate Emit® Calibrator/Control—Level 3 (200 ng/mL Cutoff), or Level 4 (300 ng/mL Cutoff)—in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the analyzer User's Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis

Prepare a calibration curve by running Emit® Calibrators/Controls Level 0 (0 ng/mL), Level 2 (100 ng/mL), Level 3 (200 ng/mL), Level 4 (300 ng/mL), and Level 5 (800 ng/mL). Validate the calibration by running controls (see Quality Control). Refer to the analyzer User's Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

Validate the calibration by assaying controls. Ensure that the result from the Emit® Calibrator/Control level (Level 0 [0 ng/mL] or Level 5 [800 ng/mL]) relates appropriately to the cutoff calibrator result from the selected cutoff calibrator level (Level 3 [200 ng/mL] or Level 4 [300 ng/mL]). Once calibration is validated, run urine specimens.

Semiquantitative Analysis

For a selected cutoff level (200 ng/mL or 300 ng/mL), validate the calibration curve by assaying commercial controls. Ensure that control results fall within acceptable limits as defined by your laboratory. Once the calibration curve is validated, run urine specimens.

Qualitative and Semiquantitative Analysis

1. Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of Quality Control (QC) material with known Barbiturate concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
2. Refer to the instrument operator's manual for appropriate instrument checks.

Evaluation and Interpretation of Results

When the Emit® II Plus Barbiturate Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given specimen cannot be estimated. The assay results distinguish between positive and negative specimens—positive indicating specimens contain barbiturates; negative indicating specimens do not contain barbiturates, or barbiturates are present in concentrations below the cutoff level for this assay.

- A specimen that gives a change in rate value equal to or higher than the rate of the selected cutoff calibrator level is interpreted as positive.
- A specimen that gives a change in rate value lower than the rate of the selected cutoff calibrator level is interpreted as negative.
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

When used semiquantitatively, the Emit® II Plus Barbiturate Assay yields the approximate concentration of the drug detected by the assay (See Section 8, Specific Performance Characteristics, Analytical Recovery). The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for confirmation by GC/MS. Semiquantitation also permits the laboratory to establish quality control procedures and assess control performance.

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

- The assay is designed for use only with human urine.
- A positive result from the assay indicates only the presence of barbiturates.
- Boric acid is not recommended as a preservative for urine.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.
- Interpretation of results must take into account that urine concentrations of barbiturates can vary extensively with fluid intake and other biological variables.
- Immunoassays that produce a single result in the presence of a drug and its metabolites cannot fully quantitate the concentration of individual components.

8 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® II Plus Barbiturate Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Positive specimens were confirmed by GC/MS. 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 refer only to reagents.

Precision

Within-run precision was determined by assaying 2 replicates of each cutoff calibrator/control and positive and negative controls twice a day for 20 days (N=80). Total precision was also calculated from these data. Table 2 summarizes the findings at the 200 ng/mL cutoff; Table 3 summarizes the findings at the 300 ng/mL cutoff.

Table 2 — Within-Run and Total Precision at 200 ng/mL

Barbiturate 200 ng/mL Cutoff	Within-Run Precision			Total Precision		
	Cutoff Cal	Control 75%	Control 125%	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	326	305	346	326	305	346
SD	3.8	3.0	3.9	5.1	4.3	6.1
%CV	1.2	1.0	1.1	1.6	1.4	1.8

Table 3 — Within-Run and Total Precision at 300 ng/mL

Barbiturate 300 ng/mL Cutoff	Within-Run Precision			Total Precision		
	Cutoff Cal	Control 75%	Control 125%	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	370	336	408	370	335	408
SD	12.1	4.1	4.6	13.7	6.1	9.1
%CV	0.8	0.5	0.5	3.7	1.8	2.2

Comparative Analysis

Clinical urine specimens were analyzed on the AU400/AU600 Clinical Chemistry System and on the SYVA®-30R Biochemical System. Specimens positive by either method contained barbiturates ranging from 302–6481 ng/mL. Table 4 summarizes the number of positive/negative results identified and the percent agreement with the SYVA®-30R Biochemical System.

Table 4 — Summary of Comparative Analysis

Assay	Positive	Negative	% Agreement
Barbiturate 200 ng/mL	70	51	95
Barbiturate 300 ng/mL	45	115	98

Analytical Recovery

Negative human urine specimens were spiked with known concentrations of secobarbital. Specimens spiked with drug concentrations lower than the cutoff concentration and tested qualitatively were correctly identified as negative 100% of the time. Specimens spiked with drug concentrations greater than the cutoff were correctly identified as positive 100% of the time. Table 5 summarizes the results on semiquantitative analysis of the specimens.

Table 5 — Semiquantitative Analysis of Barbiturate-Spiked Samples

Concentration (ng/mL)	Mean (ng/mL)
100	104
140	142
260	266
360	408
750	783

Specificity

The Emit® II Plus Barbiturate Assay detects both long- and short-acting barbiturates in human urine.

Table 6 lists the concentrations of compounds that produce a result approximately equivalent to the 200 ng/mL and 300 ng/mL calibrator/control cutoffs, respectively. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. These concentrations are within the range of the levels found in urine following use of the compound or, in the case of metabolites, the parent compound. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 6 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 6 — Concentrations (ng/mL) of Barbiturate Compounds That Produce a Result Approximately Equivalent to the 200 ng/mL and 300 ng/mL Secobarbital Cutoff

Compound	Concentration (ng/mL) at 200 ng/mL Cutoff	Concentration (ng/mL) at 300 ng/mL Cutoff
Allobarbital	345	744
Alphenal	284	354
Amobarbital	348	923
Aprobarbital	275	478
Barbital	1278	4148
5-Ethyl-5-(4-hydroxyphenyl) barbituric acid	927	4719
Butabarbital	274	523
Butalbital	304	475
Butobarbital	349	875
Cyclopentobarbital	304	527
Pentobarbital	252	447
Phenobarbital	509–971*	1600–3110*
Talbutal	194	262
Thiopental	28200	80400

*Observed Range

Table 7 lists the compounds that produce a negative result by the Emit® II Plus Barbiturate Assay. Specificity testing was performed at the 200 ng/mL cutoff, which represents the greatest potential for cross-reactivity. Positive results for compounds structurally unrelated to barbiturates have not been observed.

Table 7 — Concentrations of Compounds Showing a Negative Response

Compound	ConcentrationTested (µg/mL) at the 200 ng/mL (0.2 µg/mL) Cutoff
Acetaminophen	1000
α-Acetyl-*N,N*-dinormethadol (dinor LAAM)	25
L-α-Acetylmethadol (LAAM)	25
N-Acetylprocainamide (NAPA)	400
Acetylsalicylic acid	1000
Amitriptyline	1000
D-Amphetamine	1000
Benzoylecgonine	1000
Buprenorphine	1000
Caffeine	1000
Cimetidine	1000
Clomipramine	2.5
Clonidine	1000
Codeine	500
Cotinine	100
Cyclobenzaprine	1000
Desipramine	800
Diphenhydramine	1000
Doxepin	1000
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1000
Fluoxetine	1000
Glutethimide	300
Ibuprofen	1000
Ketamine	100
Ketorolac tromethamine	1000
Lormetazepam	1
LSD	10 ng/mL
Meperidine	1000
D-Methamphetamine	35
Methaqualone	1500
Morphine	1000

Table 7 — Concentrations of Compounds Showing a Negative Response (cont.)

Compound	ConcentrationTested (µg/mL) at the 200 ng/mL (0.2 µg/mL) Cutoff
Naproxen	1000
Nortriptyline	1000
Oxazepam	300
Phencyclidine	1000
Phenytoin	1000
Promethazine	1000
Propoxyphene	1000
Ranitidine	1000
Scopolamine	500
11-nor-Δ^9-THC-9-COOH	100
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2 mg/mL
Zolpidem	100

Non-Interfering Substances

Each of the following compounds when added to urine at ± 25% concentration of the cutoff do not yield a false response relative to the 200 ng/mL cutoff:

Table 8 — Non-Interfering Substances

Compound	Concentration
Acetone	1.0 g/dL
Ascorbic Acid	1.5 g/dL
Bilirubin	0.25 mg/dL
Creatinine	0.5 g/dL
Ethanol	1.0 g/dL
Gamma Globulin	0.5 g/dL
Glucose	2.0 g/dL
Hemoglobin	115 mg/dL
Human Serum Albumin	0.5 g/dL
Oxalic Acid	0.1 g/dL
Riboflavin	7.5 mg/dL
Sodium Chloride	6.0 g/dL
Urea	6.0 g/dL

Sensitivity

The senstivity level (minimum detection limit) of the Emit® II Plus Barbiturate Assay is 18 ng/mL. This level represents the lowest concentration of secobarbital that can be distinguished from 0 ng/mL with a confidence level of 95%.

9 REFERENCES

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2. Hofmann FE. *A Handbook on Drug and Alcohol Abuse: The Biomedical Aspects.* New York, NY: Oxford University Press; 1983.
3. Ellenhorn MJ, Barceloux DG. *Medical Toxicology.* New York, NY: Elsevier Science Publishing Company, Inc; 1988:575–580.
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5. Gorodetzky CW. Detection of drugs of abuse in biological fluids. In: Martin WR, ed. *Drug Addiction I.* New York, NY: Springer-Verlag; 1977:319–409.
6. Oellerich M. Enzyme immunoassays in clinical chemistry: present status and trends. *J Clin Chem Clin Biochem.* 1980; 18:197–208.

10 NOTES

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
	CE Mark
CONTENTS	Contents
	Reconstitution Volume
LEVEL	Level

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