Emit® II *Plus* Cannabinoid Assay

September 2013 9N052.2D_C

See shaded sections:
Updated information from August 2010 version.

Catalog Number	Product Description	Quantity/ Volume
OSR9N229	**Emit® II Plus Cannabinoid Assay**	
	OSR9N618 R1 (Antibody/Substrate Reagent 1)	2 x 31 mL
	OSR9N648 R2 (Enzyme Reagent 2)	2 x 15 mL
9A509UL	**Emit® Calibrator/Control Level 0***	1 x 14 mL
9A549UL	**Emit® Calibrator/Control Level 2 (20)***	1 x 14 mL
9A569UL	**Emit® Calibrator/Control Level 3 (50)***	1 x 14 mL
9A589UL	**Emit® Calibrator/Control Level 4 (100)***	1 x 14 mL
9A609UL	**Emit® Calibrator/Control Level 5 (200)***	1 x 14 mL

Required for calibrating the Emit® II Plus Cannabinoid 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 with the Emit® II Plus Cannabinoid Assay

Desired Cutoff Level (ng/mL)	Qualitative Analysis Required Cal/Control	Qualitative Analysis Concentration (ng/mL)	Semiquantitative Analysis Required Cal/Control	Semiquantitative Analysis Concentration (ng/mL)
20	Level 0	0	Level 0	0
	Level 2	20	Level 2	20
	Level 5	200	Level 3	50
			Level 4	100
50	Level 0	0	Level 0	0
	Level 3	50	Level 3	50
	Level 5	200	Level 4	100
			Level 5	200
100	Level 0	0	Level 0	0
	Level 4	100	Level 3	50
	Level 5	200	Level 4	100
			Level 5	200

Note: The Emit® Calibrators/Controls contain the stated concentrations of cannabinoid listed in Table 1. Emit® Calibrator/Control Levels 2, 3, 4, and 5 contain additional drugs of abuse that do not affect the assay. See the Emit® Calibrators/Controls instructions for use. For any individual cutoff level, 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.

1 INTENDED USE

The Emit® II Plus Cannabinoid Assay is a homogeneous enzyme immunoassay with a 20 ng/mL, 50 ng/mL (SAMHSA initial test cutoff level), or 100 ng/mL cutoff. The assay is intended for use in the qualitative and semiquantitative analyses of cannabinoids in human urine. These reagents are packaged specifically for use on AU® Clinical Chemistry Systems.

The Emit® II Plus Cannabinoid Assay provides only a preliminary analytical 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

Marijuana is a mixture of dried leaves and flowering tops of the plant *Cannabis sativa L*. The agents that produce the hallucinogenic and other biological effects of marijuana are called cannabinoids.

The cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive ingredient in marijuana and hashish. The compound Δ^9-THC is quickly and effectively absorbed by inhalation or from the gastrointestinal tract,[2] and is almost completely metabolized by liver enzymes.[3] Peak plasma levels of Δ^9-THC occur within 10 minutes of inhalation and approximately 1 hour after ingestion.[2] Approximately 30% of a dose of THC is excreted as urinary metabolites within 72 hours after exposure.[2] Concentration depends on the total amount of THC absorbed, frequency of abuse, rate of release from fatty tissue, and time of specimen collection with respect to use. In chronic users, THC may accumulate in fatty tissue faster than it can be eliminated. This accumulation leads to longer detection times in urinalysis for chronic users than for occasional users.[4]

The Emit® II Plus Cannabinoid Assay detects the major metabolite of Δ^9-THC, 11-nor-Δ^9-THC-9-carboxylic acid, in human urine. It also detects other Δ^9-THC metabolites. The cutoff level for distinguishing positive from negative specimens is 20 ng/mL, 50 ng/mL, or 100 ng/mL. Passive inhalation of marijuana smoke may produce positive results with low cutoff cannabinoid assays. Urine specimens from nonsmokers can test positive for cannabinoid metabolites, but only after exposure to high concentrations of marijuana smoke in a small, unventilated area. Such extreme exposure conditions clearly are not typical of usual social situations.[5] Positive results for specimens containing other compounds structurally unrelated to cannabinoids have not been observed.

Methods historically used for detecting cannabinoids in biological fluids include radioimmunoassay, gas chromatography/mass spectrometry, gas chromatography, and enzyme immunoassay.[2, 3]

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 Cannabinoid Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[3] 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:

Mouse monoclonal antibodies to Δ^9-tetrahydrocannabinol (1.2 µg/mL), G6P (5.5 mM), NAD (3.5 mM), BSA, Δ^9-tetrahydrocannabinol labeled with G6PDH (0.4 U/mL), Tris/HEPES buffer, preservatives, and stabilizers

Precautions

- For *in vitro* diagnostic use.
- Reagents 1 and 2 contain nonsterile mouse monoclonal 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 Cannabinoid 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 4.5–8.0.
- 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 Cannabinoid 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 2 (20 ng/mL Cutoff), Level 3 (50 ng/mL Cutoff), or Level 4 (100 ng/mL Cutoff)—in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the instrument User's Guide or the Application Sheet for instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis

Semiquantitative analysis is not recommended for the 20 ng/mL cutoff on the AU400®/AU600® Clinical Chemistry System. For the 50 ng/mL and 100 ng/mL cutoff levels, prepare a calibration curve by running a reagent blank (blue rack) and the Emit® Calibrators/Controls Level 3 (50 ng/mL), Level 4 (100 ng/mL), and Level 5 (200 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 relates appropriately to the cutoff calibrator result. Once calibration is validated, run urine specimens. For the 20 ng/mL cutoff, run a reagent blank (blue rack) daily to ensure consistent day-to-day control results.

Semiquantitative Analysis

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 THC 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 Cannabinoid 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 cannabinoids; negative indicating specimens do not contain cannabinoids, or cannabinoids 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.

When used semiquantitatively, the Emit® II Plus Cannabinoid Assay yields approximate, cumulative concentrations of the drugs detected by the assay (See Section 8, Specific Performance Characteristics, Analytical Recovery or Specificity). 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

- Semiquantitative analysis is not recommended for the 20 ng/mL cutoff on the AU400/AU600 Clinical Chemistry System.
- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of cannabinoids but does not indicate or measure intoxication.
- Boric acid is not recommended as a preservative for urine.
- This assay does NOT detect synthetic cannabinoids, such as JWH-018, JWH 073, etc.
- 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 concentrations of cannabinoids in urine 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 in this section is based on Emit® II Plus Cannabinoid Assay studies 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.

Precision

Within-run precision was calculated according to NCCLS Guideline EP5-A by running two 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. The following tables summarize the data (in mAU/min) for each cutoff level.

Table 2 — Within-Run Precision at the 20 ng/mL Cutoff

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	473	445	491
SD	5.9	6.1	5.2
%CV	1.2	1.4	1.1

Table 3 — Total Precision at the 20 ng/mL Cutoff

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	473	445	491
SD	12.1	10.9	11.9
%CV	2.6	2.4	2.4

Table 4 — Within-Run Precision at the 50 ng/mL Cutoff

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	482	452	498
SD	6.2	5.2	6.1
%CV	1.1	1.2	1.2

Table 5 — Total Precision at the 50 ng/mL Cutoff

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	482	452	498
SD	16.5	15.6	17.3
%CV	3.4	3.5	3.5

Table 6 — Within-Run Precision at the 100 ng/mL Cutoff (Qualitative Analysis)

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	305	281	376
SD	3.8	4.0	7.4
%CV	1.3	1.4	2.0

Table 7 — Total Precision at the 100 ng/mL Cutoff (Qualitative Analysis)

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	305	281	376
SD	5.7	7.4	16.9
%CV	1.9	2.6	4.5

Table 8 — Within-Run Precision at the 100 ng/mL Cutoff (Semiquantitative Analysis)

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	590	538	629
SD	5.4	4.0	13.2
%CV	0.9	0.7	2.1

Table 9 — Total Precision at the 100 ng/mL Cutoff (Semiquantitative Analysis)

	Cutoff Cal	Control 75%	Control 125%
Mean (mAU/min)	590	538	629
SD	18.0	19.6	17.0
%CV	3.0	3.7	2.7

Comparative Analysis

Clinical urine specimens were analyzed on the AU400/AU600 Clinical Chemistry System and on the SYVA®-30R Biochemical System. Table 10 presents the summary of GC/MS ranges (in ng/mL) observed for specimens containing 11-nor-Δ^9-THC-9-COOH; such specimens were positive by either analytical system. Table 11 summarizes the number of positive/negative results identified and the percent agreement with the SYVA®-30R Biochemical System.

Table 10 — GC/MS Ranges of Cannabinoid-Positive Specimens

	20 ng/mL Cutoff	50 ng/mL Cutoff	100 ng/mL Cutoff
GC/MS Range (ng/mL)	10.3–26.0	14.2–61.8	23.9–696.0

Table 11 — Summary of Comparative Analysis

	Positive	Negative	% Agreement
20 ng/mL Cutoff	50	50	100
50 ng/mL Cutoff	50	50	100
100 ng/mL Cutoff (Qualitative)	50	50	100
100 ng/mL Cutoff (Semiquantitative)	49	51	100

Analytical Recovery

Negative human urine specimens were spiked with concentrations of 11-nor-Δ^9-THC-9-COOH. Qualitative analysis of the specimens spiked with the drug concentrations lower than the cutoff concentration 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 12 summarizes the results on semiquantitative analysis of the specimens.

Table 12 — Semiquantitative Analysis of Cannabinoid-Spiked Samples: (50 & 100 ng/mL Cutoffs Only)

Concentration (ng/mL)	Mean (ng/mL)
25	26
35	39
70	77
150	181
200	196

Specificity

The Emit® II Plus Cannabinoid Assay detects the major metabolites of Δ^9-THC in urine.

Table 13 lists the concentrations of compounds that produce results approximately equivalent to the calibrator/control cutoffs. Each concentration represents the reactivity level for the stated compound when the compound is added to a negative urine specimen. These concentrations are within the range of the levels found in urine following use of the drug 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 13 may combine to produce a rate approximately equivalent to or greater than the cutoff calibrator.

Table 13 — Concentrations of Cannabinoids That Produce a Result Approximately Equivalent to the Various 11-nor-Δ^9-THC-9-COOH Cutoffs

Compound	20 ng/mL Cutoff	50 ng/mL Cutoff	100 ng/mL Cutoff
8-β-11-Dihydroxy-Δ^9 -THC	24	58	109
8-β-Hydroxy- Δ^9 -THC	26	68	146
11-Hydroxy- Δ^8 -THC	43	67	129
11-Hydroxy- Δ^9 -THC	42	77	124
9-Carboxy-11-nor- Δ^9 -THC-glucuronide	79	95	328

Table 14 lists the concentrations of compounds that were tested and found to give a negative response. Positive results for specimens containing other compounds structurally unrelated to cannabinoids have not been observed.

Table 14 — Concentrations of Compounds Showing Negative Response at all Cutoff Levels

Compound	Concentration Tested (µg/mL)
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	500
Ibuprofen	1000
Ketamine	100
Ketorolac Tromethamine	1000
Lormetazepam	1
LSD	10 ng/mL
Meperidine	1000
D-Methamphetamine	35
Methaqualone	1500
Morphine	1000
Naproxen	1000
Nortriptyline	1000
Oxazepam	300
Phencyclidine	1000
Phenytoin	1000
Promethazine	1000
Propoxyphene	1000
Ranitidine	1000
Secobarbital	1000

Compound	Concentration Tested (µg/mL)
Scopolamine	500
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 50 ng/mL cutoff:

Table 15 — 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 sensitivity level (minimum detection limit) of the Emit® II Plus Cannabinoid Assay using the 20 ng/mL cutoff is 14 ng/mL, and the sensitivity level for the 50 ng/mL and 100 ng/mL cutoffs is 35 ng/mL. These levels represent the lowest concentrations of 11-nor-Δ^9-THC-9-carboxylic acid that can be distinguished from 0 ng/mL with a confidence level of 95%.

9 REFERENCES

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3. Ellenhorn MJ, Barceloux DG. *Medical Toxicology.* New York, NY: Elsevier Science Publishing Company, Inc; 1988:675–682.
4. Wyngaarden JB, Smith LH Jr, eds. *Cecil Textbook of Medicine.* Philadelphia, PA: WB Saunders Co; 1986:52–58.
5. Kwong TC, et al. Critical issues in urinalysis of abused substances: report of the substance testing committee. *Clin Chem.* 1988;34/3:605–632.

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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250 S. Kraemer Blvd.
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