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Emit® II *Plus* Cannabinoid Assay

See shaded sections:
Updated information from 2015-04 version.

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Cannabinoid Assay

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),[1] or 100 ng/mL cutoff. The assay is intended for use in the qualitative and semiquantitative analyses of cannabinoids in human urine. Emit® II Plus assays are designed for use with a number of chemistry analyzers.

The Emit® II Plus Cannabinoid 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.[2] 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 and Explanation of the Test

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[3], and is almost completely metabolized by liver enzymes.[4] Peak plasma levels of Δ^9-THC occur within 10 minutes of inhalation and approximately 1 hour after ingestion.[3] Approximately 30% of a dose of THC is excreted as urinary metabolites within 72 hours after exposure.[3] 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.[5]

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.[6] 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.[3,4]

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.[2]

3 Principle

The Emit® II Plus assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[4] 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

REF	Product Description	Volume
9N039UL/	**Emit® II Plus Cannabinoid Assay**	28 mL/
9N029UL/	**Antibody/Substrate Reagent 1**	115 mL/
9N129UL	Mouse monoclonal antibodies to Δ^9-tetrahydrocannabinol (1.2 µg/mL),* bovine serum albumin, G6P (5.5 mM), NAD (3.5 mM), preservatives, and stabilizers	1000 mL
	Enzyme Reagent 2	12 mL/
	Δ^9-tetrahydrocannabinol labeled with bacterial G6PDH (0.4 U/mL),*	50 mL/
	Tris/HEPES buffer, bovine serum albumin, preservatives, and stabilizers	435 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.

Preparation and Storage of Assay Components

Reagents:

The Emit® II Plus Cannabinoid Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent bottles when not in use.

Note: Caps must always be replaced on the original containers.

When not in use, store reagents at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Refer to the analyzer-specific protocols for on instrument stability information. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C. **Improper storage of reagents can affect assay performance.**

5 Specimen Collection and Preparation

- Urine specimens may be collected in plastic (ie, polypropylene, polycarbonate, and 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 are potentially infectious.
- Baby wash/shampoo may contaminate the urine specimen taken from a neonate, causing an erroneous cannabinoid test result. Using a copious amount of water, thoroughly rinse such products from the neonate's body before collecting the specimen.

6 Procedure

Materials Provided

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

Materials Required But Not Provided

9A509UL	Emit® Calibrator/Control Level 0
9A549UL	Emit® Calibrator/Control Level 2
9A569UL	Emit® Calibrator/Control Level 3
9A589UL	Emit® Calibrator/Control Level 4
9A609UL	Emit® Calibrator/Control Level 5

Commercially available controls (see Quality Control, Semiquantitative Analysis)

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.

Daily Maintenance

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

Assay Sequence

To run the assay, see the instrument operator's manual and the analyzer-specific protocol from Siemens.

Calibration

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

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

Desired Cutoff Level (ng/mL)	Required Calibrators/Controls for Qualitative Analysis (ng/mL)	Required Calibrators/Controls for Semiquantitative Analysis (ng/mL)
20	Level 0 (0) Level 2 (20) Level 5 (200)	Level 0 (0) Level 2 (20) Level 3 (50) Level 4 (100)
50	Level 0 (0) Level 3 (50) Level 5 (200)	Level 0 (0) Level 3 (50) Level 4 (100)
100	Level 0 (0) Level 4 (100) Level 5 (200)	Level 5 (200)

Note: 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.

Qualitative Analysis

20 ng/mL CUTOFF

Run the Emit® Calibrator/Control Level 2 (20 ng/mL) in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the analyzer-specific protocol for additional information and instrument settings. Recalibrate as indicated by control results.

50 ng/mL CUTOFF

Run the Emit® Calibrator/Control Level 3 (50 ng/mL) in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the analyzer-specific protocol for additional information and instrument settings. Recalibrate as indicated by control results.

100 ng/mL CUTOFF

Run the Emit® Calibrator/Control Level 4 (100 ng/mL) in duplicate. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the analyzer-specific protocol for additional information and 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 (20 ng/mL), 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 Emit® Calibrators/Controls instructions for use and the analyzer-specific protocol for additional information and instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

20 ng/mL CUTOFF

Validate the calibration by assaying controls. Ensure that the result from Emit® Calibrator/Control Level 0 (0 ng/mL) or Emit® Calibrator/Control Level 5 (200 ng/mL) relates appropriately to the result from Emit® Calibrator/Control Level 2 (20 ng/mL). That is,

- If Emit® Calibrator/Control Level 0 (0 ng/mL) was run, ensure that the result is negative relative to Emit® Calibrator/Control Level 2 (20 ng/mL).
- If Emit® Calibrator/Control Level 5 (200 ng/mL) was run, ensure that the result is positive relative to Emit® Calibrator/Control Level 2 (20 ng/mL).

Once the calibration is validated, run urine specimens.

50 ng/mL CUTOFF

Validate the calibration by assaying controls. Ensure that the result from Emit® Calibrator/Control Level 0 (0 ng/mL) or Emit® Calibrator/Control Level 5 (200 ng/mL) relates appropriately to the result from Emit® Calibrator/Control Level 3 (50 ng/mL). That is,

- If Emit® Calibrator/Control Level 0 (0 ng/mL) was run, ensure that it tests negative relative to Emit® Calibrator/Control Level 3 (50 ng/mL).
- If Emit® Calibrator/Control Level 5 (200 ng/mL) was run, ensure that it tests positive relative to Emit® Calibrator/Control Level 3 (50 ng/mL).

Once the calibration is validated, run urine specimens.

100 ng/mL CUTOFF

Validate the calibration by assaying controls. Ensure that the result from Emit® Calibrator/Control Level 0 (0 ng/mL) or Emit® Calibrator/Control Level 5 (200 ng/mL) relates appropriately to the result from Emit® Calibrator/Control Level 4 (100 ng/mL). That is,

- If Emit® Calibrator/Control Level 0 (0 ng/mL) was run, ensure that it tests negative relative to Emit® Calibrator/Control Level 4 (100 ng/mL).
- If Emit® Calibrator/Control Level 5 (200 ng/mL) was run, ensure that it tests positive relative to Emit® Calibrator/Control Level 4 (100 ng/mL).

Once the calibration is validated, run urine specimens.

Semiquantitative Analysis

20 ng/mL CUTOFF

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.

50 ng/mL CUTOFF

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.

100 ng/mL CUTOFF

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.

7 Results

Qualitative Analysis

20 ng/mL

The Emit® Calibrator/Control Level 2, which contains a concentration of 20 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid, is used as a reference for distinguishing between "positive" and "negative" specimens.

Positive Results. A specimen that gives a change in rate value equal to or higher than the Emit® Calibrator/Control Level 2 rate value is interpreted as positive: The specimen contains cannabinoids.

Negative Results. A specimen that gives a change in rate value lower than the Emit® Calibrator/Control Level 2 rate value is interpreted as negative: Either the specimen does not contain cannabinoids or cannabinoids are present in concentrations below the cutoff level for this assay.

50 ng/mL

The Emit® Calibrator/Control Level 3, which contains a concentration of 50 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid, is used as a reference for distinguishing "positive" from "negative" specimens.

Positive Results. A specimen that gives a change in rate value equal to or higher than the Emit® Calibrator/Control Level 3 rate value is interpreted as positive: The specimen contains cannabinoids.

Negative Results. A specimen that gives a change in rate value lower than the Emit® Calibrator/Control Level 3 rate value is interpreted as negative: Either the specimen does not contain cannabinoids or cannabinoids are present in concentrations below the cutoff level for this assay.

100 ng/mL

The Emit® Calibrator/Control Level 4, which contains a concentration of 100 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid, is used as a reference for distinguishing "positive" from "negative" specimens.

Positive Results. A specimen that gives a change in rate value equal to or higher than the Emit® Calibrator/Control Level 4 rate value is interpreted as positive: The specimen contains cannabinoids.

Negative Results. A specimen that gives a change in rate value lower than the Emit® Calibrator/Control Level 4 rate value is interpreted as negative: Either the specimen does not contain cannabinoids or cannabinoids are present in concentrations below the cutoff level for this assay.

Semiquantitative Analysis

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. Refer to the Analytical Recovery section for the semiquantitative range.

Using the Emit® II Plus Cannabinoid Assay, it is possible to make semiquantitative determinations of cannabinoids. See Table 1, Emit® Calibrators/Controls for Use in Qualitative or Semiquantitative Analysis, for requirements. Refer to the analyzer-specific protocol for further instructions.

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

- 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 urine concentrations 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.

9 Expected Values

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 positive from negative specimens—positive indicating specimens that contain cannabinoids.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drugs detected by the assay (see Section 7, Results).

10 Specific Performance Characteristics

The data appearing in this section were collected on the SYVA®-30R Biochemical System using the Emit® II Plus Cannabinoid Assay and the appropriate Emit® II Cannabinoid Assay (comparative method). Positive specimens were confirmed by GC/MS.

20 ng/mL CUTOFF

Accuracy

Qualitative Results

One hundred (100) specimens were analyzed by the Emit® II Plus Cannabinoid Assay and by the comparative method.

Both the Emit® II Plus Cannabinoid Assay and the comparative method use an optional cutoff level of 20 ng/mL for distinguishing positive results from negative results.

Fifty (50) specimens showed positive results by both methods, and 50 specimens showed negative results by both methods.

All specimens that showed positive results by either method were confirmed by GC/MS to contain cannabinoid concentrations above the limit of quantitation (LOQ). The LOQ of the GC/MS method was 2 ng/mL. Analysis by GC/MS confirmed the positive specimens contained between 2 ng/mL and greater than 100 ng/mL cannabinoids.

No drug was detected by GC/MS in 20 negative specimens that were randomly selected from the 50 negative specimens.

Data are summarized in Table 2.

Table 2 — Accuracy of Qualitative Results for the 20 ng/mL Cutoff

Emit® II Plus Cannabinoid Assay		Comparative Method +	Comparative Method −
Emit® II Plus Cannabinoid Assay	+	50	0
	−	0	50

Analytical Recovery

Qualitative Results

The Emit® II Plus Cannabinoid Assay correctly identified the mean rate of spiked specimens containing less than 20 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as negative and the mean rate of spiked specimens containing greater than 20 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as positive.

Semiquantitative Results

Negative human urine specimens were spiked with concentrations of 11-nor-Δ^9-THC-9-carboxylic acid at levels throughout the semiquantitative range of 15 to 55 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Cannabinoid Assay. Semiquantitative results are shown in Table 3.

Table 3 — Accuracy by Analytical Recovery of Semiquantitative Results for the 20 ng/mL Cutoff

Nominal 11-nor-Δ^9-THC-9-COOH Concentration (ng/mL)	Average 11-nor-Δ^9-THC-9-COOH Concentration by Emit® II Plus Assay (ng/mL)	Recovery (%)
15	16	106
18	17.5	97
22	19.8	90
25	21.1	84
30	23.7	79
55	50.8	92

Precision

Qualitative analysis of precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Semiquantitative precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 4 and 5.

Table 4 — Qualitative Analysis of Precision for the 20 ng/mL Cutoff

Calibrator or Control	Mean (mAU/min)	SD	CV (%)
Within-Run Precision			
0 ng/mL Calibrator	233.1	1.6	0.7
Control Level 1 (15 ng/mL)	271.4	2.1	0.8
20 ng/mL Calibrator	288.2	1.4	0.5
Control Level 2 (25 ng/mL)	299.6	2	0.7
Total Precision			
0 ng/mL Calibrator	233.1	3.6	1.5
Control Level 1 (15 ng/mL)	271.4	5.6	2.1
20 ng/mL Calibrator	288.2	3.9	1.4
Control Level 2 (25 ng/mL)	299.6	7.9	2.6

Table 5 — Semiquantitative Analysis of Precision for the 20 ng/mL Cutoff

Calibrator or Control	Mean (ng/mL)	SD	CV (%)
Within-Run Precision			
Control Level 1 (15 ng/mL)	17.2	0.4	2.3
20 ng/mL Calibrator	20.2	0.4	2
Control Level 2 (25 ng/mL)	22.2	0.4	1.8
Total Precision			
Control Level 1 (15 ng/mL)	17.2	1.1	6.4
20 ng/mL Calibrator	20.2	1	4.9
Control Level 2 (25 ng/mL)	22.2	1.5	6.7

Specificity

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

Table 6 gives the compounds this assay detects and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator/Control Level 2, 20 ng/mL). These concentrations are within the range of levels found in urine following the use of the drug, or in the case of metabolites, the parent compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. 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 — Concentration of Cannabinoids That Produce a Result Approximately Equivalent to the 20 ng/mL 11-nor-Δ^9-THC-9-COOH Cutoff

Compound	Concentration (ng/mL)
8-ß-11-Dihydroxy-Δ^9-THC	24
8-ß-Hydroxy-Δ^9-THC	26
11-Hydroxy-Δ^8-THC	43
11-Hydroxy-Δ^9-THC	42
9-Carboxy-11-nor-Δ^9-THC-glucuronide	79
Δ^8-THC	79
Δ^9-THC	78

Table 7 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 7 — Concentrations of Compounds Showing Negative Response at a 20 ng/mL Cutoff

Compound	Concentration Tested (µg/mL) at the 20 ng/mL (0.02 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	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
Scopolamine	500
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2 mg/mL
Zolpidem	100

Sensitivity

The sensitivity level of the Emit® II Plus Cannabinoid Assay using the 20 ng/mL cutoff is less than 10 ng/mL. This level represents the lowest concentration of 11-nor-Δ^9-THC-9-carboxylic acid that can be distinguished from 0 ng/mL with a confidence level of 95%.

50 ng/mL CUTOFF

Accuracy

Qualitative Results

One hundred (100) specimens were analyzed by the Emit® II Plus Cannabinoid Assay and by the comparative method.

Both the Emit® II Plus Cannabinoid Assay and the comparative method use a cutoff level of 50 ng/mL (SAMHSA initial test cutoff level) for distinguishing between positive results and negative results.

Fifty (50) specimens showed positive results by both methods, and 50 specimens showed negative results by both methods.

All specimens that showed positive results by either method were confirmed by GC/MS to contain cannabinoids. Forty-eight (48) of the positive specimens were confirmed by GC/MS to contain cannabinoids between 15 ng/mL (SAMHSA confirmatory cutoff level) and greater than 100 ng/mL. Two (2) of the positive specimens contained cannabinoid concentrations of 14.2 ng/mL as determined by GC/MS.

No drug was detected by GC/MS in 20 negative specimens that were randomly selected from the 50 negative specimens.

Data are summarized in Table 8.

Table 8 — Accuracy of Qualitative Results for the 50 ng/mL Cutoff

Emit® II Plus Cannabinoid Assay		Comparative Method	
		+	−
Emit® II Plus Cannabinoid Assay	+	50	0
	−	0	50

Analytical Recovery

Qualitative Results

The Emit® II Plus Cannabinoid Assay correctly identified the mean rate of spiked specimens containing less than 50 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as negative and the mean rate of spiked specimens containing greater than 50 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as positive.

Semiquantitative Results

Negative human urine specimens were spiked with concentrations of 11-nor-Δ^9-THC-9-carboxylic acid at levels throughout the semiquantitative range of 25 to 180 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Cannabinoid Assay. Semiquantitative results are shown in Table 9.

Table 9 — Accuracy by Analytical Recovery of Semiquantitative Results for the 50 ng/mL Cutoff

Nominal 11-nor-Δ^9-THC-9-COOH Concentration (ng/mL)	Average 11-nor-Δ^9-THC-9-COOH Concentration by Emit® II Plus Assay (ng/mL)	Recovery (%)
25	30	118
30	33	110
37.5	39	104
45	42	94
50	45	90
55	48	87
75	65	86
125	130	104
150	163	109
180	179	99

Precision

Qualitative analysis of precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Semiquantitative precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 10 and 11.

Table 10 — Qualitative Analysis of Precision for the 50 ng/mL Cutoff

Calibrator or Control	Mean (mAU/min)	SD	CV (%)
Within-Run Precision			
0 ng/mL Calibrator	237.8	1.9	0.8
Control Level 1 (37.5 ng/mL)	299.3	2	0.7
50 ng/mL Calibrator	329.5	2.2	0.7
Control Level 2 (62.5 ng/mL)	339.1	2.1	0.6
Total Precision			
0 ng/mL Calibrator	237.8	3.6	1.5
Control Level 1 (37.5 ng/mL)	299.3	6.2	2.1
50 ng/mL Calibrator	329.5	4.7	1.4
Control Level 2 (62.5 ng/mL)	339.1	4.9	1.4

Table 11 — Semiquantitative Analysis of Precision for the 50 ng/mL Cutoff

Calibrator or Control	Mean (ng/mL)	SD	CV (%)
Within-Run Precision			
Control Level 1 (37.5 ng/mL)	40.4	0.6	1.5
50 ng/mL Calibrator	49.4	0.7	1.4
Control Level 2 (62.5 ng/mL)	52.6	0.7	1.3
Total Precision			
Control Level 1 (37.5 ng/mL)	40.4	2.2	5.4
50 ng/mL Calibrator	49.4	1.4	2.8
Control Level 2 (62.5 ng/mL)	52.6	1.9	3.6

Specificity

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

Table 12 gives the compounds this assay detects and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator/Control Level 3, 50 ng/mL). These concentrations are within the range of levels found in urine following the use of the drug, or in the case of metabolites, the parent compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 12 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 12 — Concentration of Cannabinoids That Produce a Result Approximately Equivalent to the 50 ng/mL 11-nor-Δ^9-THC-9-COOH Cutoff

Compound	Concentration (ng/mL)
8-ß-11-Dihydroxy-Δ^9-THC	58
8-ß-Hydroxy-Δ^9-THC	68
11-Hydroxy-Δ^8-THC	67
11-Hydroxy-Δ^9-THC	77
9-Carboxy-11-nor-Δ^9-THC-glucuronide	95
Δ^8-THC	220
Δ^9-THC	220

Table 13 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 13 — Concentrations of Compounds Showing Negative Response at a 50 ng/mL Cutoff

Compound	Concentration Tested (µg/mL) at the 50 ng/mL (0.05 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
Naproxen	1000
Nortriptyline	1000
Oxazepam	300
Phencyclidine	1000
Phenytoin	1000
Promethazine	1000
Propoxyphene	1000
Ranitidine	1000
Secobarbital	1000
Scopolamine	500
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2 mg/mL
Zolpidem	100

Sensitivity

The sensitivity level of the Emit® II Plus Cannabinoid Assay using the 50 ng/mL cutoff is less than 15 ng/mL. This level represents the lowest concentration of 11-nor-Δ^9-THC-9-carboxylic acid that can be distinguished from 0 ng/mL with a confidence level of 95%.

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 14 — 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

100 ng/mL CUTOFF

Accuracy

Qualitative Results

One hundred (100) specimens were analyzed by the Emit® II Plus Cannabinoid Assay and by the comparative method.

Both the Emit® II Plus Cannabinoid Assay and the comparative method use an optional cutoff level of 100 ng/mL for distinguishing between positive results and negative results.

Forty-nine (49) specimens showed positive results by both methods, and 50 specimens showed negative results by both methods.

All specimens that showed positive results by either method were confirmed by GC/MS to contain cannabinoid concentrations above the limit of quantitation (LOQ). The LOQ of the GC/MS method was 2 ng/mL. Analysis by GC/MS confirmed the positive specimens contained between 15 ng/mL and greater than 100 ng/mL cannabinoids.

One specimen showed a negative result by the Emit® II Plus Cannabinoid Assay and a positive result by the comparative method. This specimen contained 47.1 ng/mL 11-nor-Δ^9-THC-9-COOH metabolites as determined by GC/MS.

No drug was detected by GC/MS in 20 negative specimens that were randomly selected from the 50 negative specimens.

Data are summarized in Table 15.

Table 15 — Accuracy of Qualitative Results for the 100 ng/mL Cutoff

Emit® II Plus Cannabinoid Assay		Comparative Method +	Comparative Method −
Emit® II Plus Cannabinoid Assay	+	49	0
	−	1*	50

Shown to contain 47.1 ng/mL 11-nor-Δ^9-THC-9-COOH metabolites as determined by GC/MS.

Analytical Recovery

Qualitative Results

The Emit® II Plus Cannabinoid Assay correctly identified the mean rate of spiked specimens containing less than 100 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as negative and the mean rate of spiked specimens containing greater than 100 ng/mL 11-nor-Δ^9-THC-9-carboxylic acid as positive.

Semiquantitative Results

Negative human urine specimens were spiked with concentrations of 11-nor-Δ^9-THC-9-carboxylic acid at levels throughout the semiquantitative range of 15 to 180 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Cannabinoid Assay. Semiquantitative results are shown in Table 16.

Table 16 — Accuracy by Analytical Recovery of Semiquantitative Results for the 100 ng/mL Cutoff

Nominal 11-nor-Δ^9-THC-9-COOH Concentration (ng/mL)	Average 11-nor-Δ^9-THC-9-COOH Concentration by Emit® II Plus Assay (ng/mL)	Recovery(%)
15	12	80
45	36	80
50	41	83
75	62	82
90	74	82
100	95	95
125	110	88
150	153	102
180	192	106

Precision

Qualitative analysis of precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Semiquantitative precision was determined by assaying calibrators and controls on 20 days, 2 runs per day in replicates of 3 (N = 120). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 17 and 18.

Table 17 — Qualitative Analysis of Precision for the 100 ng/mL Cutoff

Calibrator or Control	Mean (mAU/min)	SD	CV (%)
Within-Run Precision			
0 ng/mL Calibrator	241.8	1.8	0.7
Control Level 1 (75 ng/mL)	316.6	2.3	0.7
100 ng/mL Calibrator	347.5	2.1	0.6
Control Level 2 (125 ng/mL)	371.5	2.3	0.6
Total Precision			
0 ng/mL Calibrator	241.8	3.2	1.3
Control Level 1 (75 ng/mL)	316.6	5.5	1.7
100 ng/mL Calibrator	347.5	5	1.4
Control Level 2 (125 ng/mL)	371.5	7.6	2

Table 18 — Semiquantitative Analysis of Precision for the 100 ng/mL Cutoff

Calibrator or Control	Mean (ng/mL)	SD	CV (%)
Within-Run Precision			
Control Level 1 (75 ng/mL)	70.3	1.9	2.7
100 ng/mL Calibrator	98.1	2.1	2.1
Control Level 2 (125 ng/mL)	125.2	2.9	2.3
Total Precision			
Control Level 1 (75 ng/mL)	70.3	5.2	7.4
100 ng/mL Calibrator	98.1	5.9	6
Control Level 2 (125 ng/mL)	125.2	8.3	6.6

Specificity

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

Table 19 gives the compounds this assay detects and the levels at which the compounds have been found to give a response approximately equivalent to that of the cutoff calibrator (Emit® Calibrator/Control Level 4, 100 ng/mL). These concentrations are within the range of levels found in urine following the use of the drug, or in the case of metabolites, the parent compound. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. If a specimen contains more than one compound detected by the assay, lower concentrations than those listed in Table 19 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 19 — Concentration of Cannabinoids That Produce a Result Approximately Equivalent to the 100 ng/mL 11-nor-Δ^9-THC-9-COOH Cutoff

Compound	Concentration (ng/mL)
8-ß-11-Dihydroxy-Δ^9-THC	109
8-ß-Hydroxy-Δ^9-THC	146
11-Hydroxy-Δ^8-THC	129
11-Hydroxy-Δ^9-THC	124
9-Carboxy-11-nor-Δ^9-THC-glucuronide	328
Δ^8-THC	660
Δ^9-THC	620

Table 20 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 20 — Concentrations of Compounds Showing Negative Response at a 100 ng/mL Cutoff

Compound	Concentration Tested (µg/mL) at the 100 ng/mL (0.1 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	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
Scopolamine	500
Thioridazine	100
Tramadol	1000
Tyramine	100
Zidovudine (AZT)	2 mg/mL
Zolpidem	100

Sensitivity

The sensitivity level of the Emit® II Plus Cannabinoid Assay using the 100 ng/mL cutoff is less than 15 ng/mL. This level represents the lowest concentration of 11-nor-Δ^9-THC-9-carboxylic acid that can be distinguished from 0 ng/mL with a confidence level of 95%.

11 Risk and Safety

H317
P280, P272, P302 + P352, P333 + P313, P501
Warning!
May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

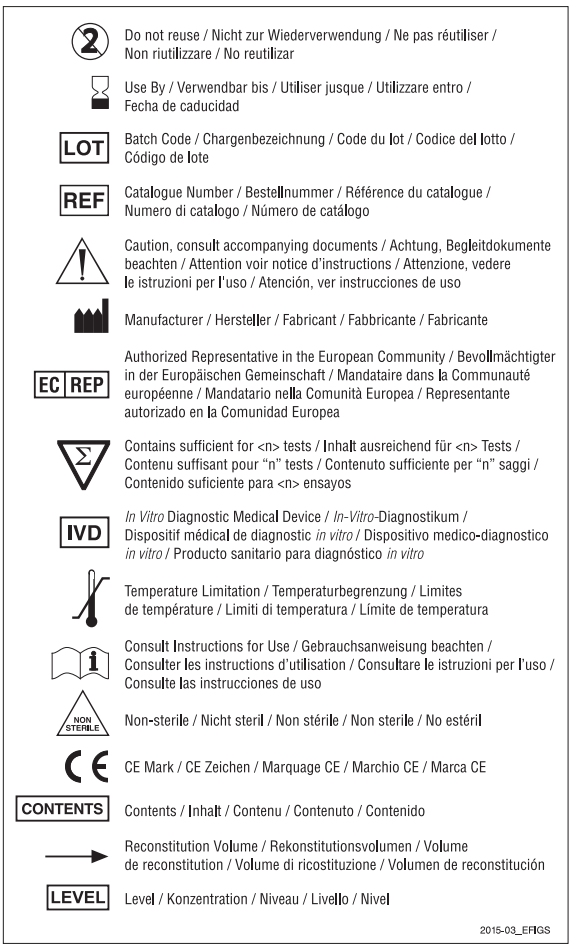
Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

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

13 Bibliography

1. Mandatory Guidelines for Federal Workplace Drug Testing Programs. Federal Register/Volume 73, No. 228, Effective October 1, 2010. pp 71858-71907.
2. Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse.* Rockville, MD: National Institute on Drug Abuse (NIDA), Department of Health and Human Services; 1986. NIDA research monograph 73.
3. Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemical in Man.* 3rd ed. Chicago, IL: Year Book Medical Publishers Inc; 1990:780–783.
4. Ellenhorn MJ, Barceloux DG. *Medical Toxicology.* New York, NY: Elesevier Science Publishing Company Inc; 1988:675–682.
5. Wyngaarden JB, Smith LH Jr, eds. *Cecil Textbook of Medicine.* Philadelphia, PA: WB Saunders Co; 1986:52–58.
6. Kwong TC, et al. Critical issues in urinalysis of abused substances: report of the substance testing committee. *Clin Chem.* 1988; 34/3:605–632.

14 Symbols Key



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