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Syva®

EMIT® II Plus Amphetamines Assay

Shading indicates technical content that differs from the previous version.

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Amphetamines Assay

1 Intended Use

The Emit® II Plus Amphetamines Assay is a homogeneous enzyme immunoassay with a 300 ng/mL, 500 ng/mL (SAMHSA initial test cutoff level) or 1000 ng/mL cutoff.[1] The assay is intended for use in the qualitative and semiquantitative analyses of amphetamines in human urine. Emit® II Plus assays are designed for use with a number of chemistry analyzers.

The Emit® II Plus Amphetamines 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

Measurements obtained with the AMPH method are an aid to diagnosis of amphetamines use. A more specific alternative chemical method must be used to obtain a confirmed analytical result.

Amphetamines are central nervous system stimulants that produce wakefulness, alertness, increased energy, reduced hunger, and an overall feeling of well being. The term "amphetamines" refers to a group of drugs that includes d-amphetamine, d-methamphetamine (*N*-methyl derivative of amphetamine), d,l-amphetamine, methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA).[3] Amphetamines can be inhaled, taken orally, intravenously, or by smoking.[3]

Amphetamines are readily absorbed from the gastrointestinal tract and are then either deactivated by the liver or excreted unchanged in the urine. The relative importance of these elimination modes depends on urinary pH. Amphetamine is metabolized to deaminated (hippuric and benzoic acids) and hydroxylated metabolites. Methamphetamine is partially metabolized to amphetamine, its major active metabolite.[3]

Amphetamines appear in urine within three hours after any type of administration[4] and can be detected by this Emit® assay for as long as 24–48 hours after the last dose.[2] The Emit® II Plus Amphetamines Assay is an automated assay that detects both d-amphetamine and d-methamphetamine. The assay also detects d,l amphetamine, d,l methamphetamine, l-amphetamine, l-methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDEA) in human urine (see Table 14). The assay contains monoclonal antibodies and is therefore less subject to interferences from amphetamine-like compounds than assays containing polyclonal antibodies. While interferences are reduced with this assay, like any immunological test, some interfering compounds do exist. For this reason, confirmation of preliminary positive results is always recommended.

Methods historically used for detecting amphetamines in biological fluids include liquid chromatography, gas-liquid chromatography, fluorometry, and enzyme immunoassay.[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.[2]

3 Principle

The Emit® II Plus Amphetamines 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

REF	Product Description	Volume
9C039UL/ 9C309UL/ 9C329UL	**Emit® II Plus Amphetamines Assay Antibody/Substrate Reagent 1** Mouse monoclonal antibodies to d-amphetamine (61 µg/mL) and d-methaphetamine (10 µg/mL),* bovine serum albumin, G6P (5.5 mM), NAD (3.5 mM), preservatives, and stabilizers	29 mL/ 115 mL/ 1000 mL
	Enzyme Reagent 2 Amphetamines labeled with bacterial G6PDH (0.72 U/mL),* Tris buffer, bovine serum albumin, preservatives, and stabilizers	12 mL/ 50 mL/ 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.

Risk and Safety

Safety data sheets (SDS/SDS) available on siemens-healthineers.com

Contain 2-methyl-2H-isothiazol-3-one hydrochloride. May produce an allergic reaction.

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Precautions

Contains sodium azide (<0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

For Professional Use.

Caution: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

For *in vitro* diagnostic use.

The Emit® II Plus Amphetamines Assay reagents are provided liquid, 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, reagents must be stored 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 application sheet for on-instrument stability information. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

5 Specimen Collection and Preparation

- Urine specimens may be collected in plastic or glass containers.
- 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.
- Urine specimens within the pH range of 3 to 11 do not require prior pH adjustment.
- 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 Amphetamines Assay
Antibody/Substrate Reagent 1
Enzyme Reagent 2

Materials Required But Not Provided

For calibration of the assay and as quality control for Qualitative Analysis:

9A509UL	Emit Calibrator/Control Level 0
9A529UL	Emit Calibrator/Control Level 1
9A549UL	Emit Calibrator/Control Level 2
9A569UL	Emit Calibrator/Control Level 3
9A609UL	Emit Calibrator/Control Level 5

Commercially available controls

The use of d-amphetamine controls with the 1000 ng/mL cutoff is not recommended. Recovery and performance of d-amphetamine and d-methamphetamine are not equivalent above 1100 ng/mL.

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.

Assay Sequence

To run the assay, see the instrument operator's manual and the application sheets available from Siemens.

Calibration

Note: These reagents are qualified for use with Emit® Calibrators/Controls only. However, other control material may be used for quality control purposes.

Table 1 — d-Methamphetamine Concentrations in Emit® Calibrators/Controls for Use in Qualitative or Semiquantitative Analysis

Cutoff Calibrator/Control (ng/mL)	Additional Recommended Calibrators/Controls for Qualitative Analysis (ng/mL)	Required Calibrators/Controls for Semiquantitative Analysis (ng/mL)
300 (Level 1)	Level 0 (0) Level 5 (2000)	Level 0 (0) Level 1 (300) Level 2 (500) Level 3 (1000)
500 (Level 2)	Level 0 (0) Level 5 (2000)	Level 0 (0) Level 1 (300) Level 2 (500)
1000 (Level 3)	Level 0 (0) Level 5 (2000)	Level 3 (1000) Level 5 (2000)

Note: For any individual cutoff level, a calibrator/control is used 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

Calibrate by running the appropriate Emit® Calibrator/Control Level for the desired cutoff listed in Table 1. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the application sheet for additional information and instrument settings. Recalibrate as indicated by control results.

Semiquantitative Analysis

Prepare a calibration curve by running the appropriate Emit® Calibrators/Controls listed in Table 1. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Calibrators/Controls instructions for use and the application sheet for additional information and instrument settings. Recalibrate as indicated by control results.

Quality Control

Qualitative Analysis

Refer to Table 1 for the desired 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 (2000 ng/mL) relates appropriately to the result from the cutoff calibrator. That is,

- If Emit® Calibrator/Control Level 0 (0 ng/mL) was run, ensure that the result is negative relative to the selected cutoff calibrator level.
- If Emit® Calibrator/Control Level 5 (2000 ng/mL) was run, ensure that the result is positive relative to the selected cutoff calibrator level.

Once the calibration is validated, run urine specimens.

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

At least once each day of use, analyze two levels of Quality Control (QC) material with known Methamphetamine or Amphetamine concentrations. For assistance in identifying a quality control material, refer to *Syva Drug Testing Quality Control Material Supplement* available on siemens-healthineers.com.

Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

In addition, perform quality control:

- Following a valid calibration
- With use of a new lot of reagent
- When troubleshooting test results that do not match clinical conditions or symptoms

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system operating instructions.

7 Results

Qualitative Analysis

Refer to Table 1 for the appropriate cutoff Emit® Calibrator/Control. Table 1 contains the concentration of d-methamphetamine present in the Emit® Calibrator/Control selected as a cutoff for distinguishing "positive" from "negative" specimens.

Positive Results. A specimen that gives a change in rate value greater than or equal to the Emit® Calibrator/Control cutoff rate value is interpreted as positive.

Negative Results. A specimen that gives a change in rate value less than the Emit® Calibrator/Control cutoff rate value is interpreted as negative: Either the specimen does not contain amphetamines or amphetamines 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 Amphetamines Assay, it is possible to make semiquantitative determinations of amphetamines. An estimate of relative total drug concentrations may be obtained by running the appropriate Emit® Calibrators/Controls: Levels 0 (0 ng/mL), 1 (300 ng/mL), 2 (500 ng/mL), 3 (1000 ng/mL), 5 (2000 ng/mL). Refer to the application sheet for 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 amphetamines but does not indicate or measure intoxication.
- There is a possibility that substances and/or factors not listed (e.g., 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.
- Boric acid is not recommended as a preservative for urine.

9 Expected Values

When the Emit® II Plus Amphetamines 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 that contain amphetamines.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drugs or drug metabolites 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 Amphetamines Assay. Different sample sizes were used to collect the data in the 300 cutoff subsection versus the 500 and 1000 ng/mL cutoff subsections. See the application sheet

for each analyzer for appropriate sample sizes. The urine specimens for the 1000 ng/mL cutoff were also assayed using the Emit® II Plus Monoclonal Amphetamine/Methamphetamine Assay (comparative method). All samples positive and negative were analyzed by GC/MS.

300 ng/mL Cutoff

Accuracy

Qualitative Results

One hundred twenty-four (124) specimens were analyzed by the Emit® II Plus Amphetamines Assay and the reference method (GC/MS). Sixty-two (62) specimens showed positive results by both methods and 58 samples showed negative results by both methods. Data are summarized in Tables 2 and 3. All positive specimens with both methods were positive by GC/MS according to our guideline of amphetamine plus methamphetamine ≥300 ng/mL.

Table 2 — Accuracy of Qualitative Results for the 300 ng/mL Cutoff

		GC/MS +	GC/MS −
Emit® II Plus Amphetamines Assay	+	62	0
	−	4	58

Table 3 — Discrepant Results

Sample Rate (mAU/min)	GC/MS (ng/mL) Amphetamine	GC/MS (ng/mL) Methamphetamine
380	9	311
400	70	275
390	67	255
407	131	214

Cutoff calibrator rate (mAU/min) = 411

Analytical Recovery

Qualitative Results

In qualitative spike analysis, the Emit® II Plus Amphetamines Assay correctly identified the mean rate of spiked specimens containing less than 300 ng/mL of d-amphetamine or d-methamphetamine as negative, and the mean rate of spiked specimens containing greater than 300 ng/mL of d-amphetamine or d-methamphetamine as positive.

Semiquantitative Results

Negative human urine was spiked with concentrations of d-amphetamine at levels throughout the semiquantitative range of 100 to 625 ng/mL. Negative human urine was also spiked with concentrations of d-methamphetamine at levels throughout the semiquantitative range of 150 to 1000 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Amphetamines Assay. Semiquantitative results are shown in Tables 4 and 5.

Table 4 — d-Amphetamine Spiked Sample Semiquantitative Analysis (300 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
100	114	114
200	194	97
250	239	95
330	323	98
500	543	109
625	711	114

Table 5 — d-Methamphetamine Spiked Sample Semiquantitative Analysis (300 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
150	158	105
225	214	95
270	259	96
330	315	96
500	513	103
750	726	97
1000	1002	100

Precision

Precision was determined by assaying calibrators and controls for 20 days, 2 runs per day in replicates of 2 (N = 80). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 6 and 7.

Table 6 — Qualitative Analysis of Precision (300 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
225	380	1.6	0.4	225	380	1.7	0.4
300	410	1.0	0.2	300	414	1.2	0.3
375	444	1.2	0.3	375	449	1.4	0.3
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
225	380	2.1	0.5	225	380	2.7	0.7
300	410	1.7	0.4	300	414	1.9	0.5
375	444	1.7	0.4	375	449	2.1	0.5

Table 7 — Semiquantitative Analysis of Precision (300 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
225	243	1.9	0.8	225	243	2.7	1.1
300	298	2.0	0.7	300	306	2.5	0.8
375	380	3.5	0.9	375	395	4.4	1.1
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
225	243	3.1	1.3	225	243	4.5	1.8
300	298	3.3	1.1	300	306	3.8	1.3
375	380	5.1	1.4	375	395	6.8	1.7

500 ng/mL Cutoff

Accuracy

Qualitative Results

One hundred twenty-four (124) specimens were analyzed by the Emit® II Plus Amphetamines Assay and the reference method (GC/MS). Fifty-eight (58) specimens showed positive results by both methods and 61 samples showed negative results by both methods. Data are summarized in Tables 8 and 9. All positive specimens with both methods were positive by GC/MS according to the new proposed SAMHSA confirmatory guidelines by the following criteria: ≥250 ng/mL methamphetamine and ≥100 ng/mL amphetamine or ≥250 ng/mL amphetamine regardless of the methamphetamine concentration.[7]

Table 8 — Accuracy of Qualitative Results for the 500 ng/mL Cutoff

		GC/MS +	GC/MS −
Emit® II Plus Amphetamines Assay	+	58	4
	−	1	61

Table 9 — Discrepant Results

Sample Rate (mAU/min)	GC/MS (ng/mL) Amphetamine	GC/MS (ng/mL) Methamphetamine
431	64	598
428	48	528
420	50	577
421	63	556
406	245	421

Cutoff calibrator rate (mAU/min) = 419

Analytical Recovery

Qualitative Results

In qualitative spike analysis, the Emit® II Plus Amphetamines Assay correctly identified the mean rate of spiked specimens containing less than 500 ng/mL of d-amphetamine or d-methamphetamine as negative, and the mean rate of spiked specimens containing greater than 500 ng/mL of d-amphetamine or d-methamphetamine as positive.

Semiquantitative Results

Negative human urine was spiked with concentrations of d-amphetamine at levels throughout the semiquantitative range of 150 to 1100 ng/mL. Negative human urine was also spiked with concentrations of d-methamphetamine at levels throughout the semiquantitative range of 200 to 1800 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Amphetamines Assay. Semiquantitative results are shown in Tables 10 and 11.

Analytical Recovery of Semiquantitative Results

Table 10 — d-Amphetamine Spiked Sample Semiquantitative Analysis (500 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
150	168	112
225	219	97
300	275	92
450	441	98
550	537	98
750	795	106
900	959	107
1100	1200	109

Table 11 — d-Methamphetamine Spiked Sample Semiquantitative Analysis (500 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
200	210	105
250	243	97
330	309	94
500	498	100
750	743	99
1100	1033	94
1500	1443	96
1800	1712	95

Precision

Precision was determined by assaying calibrators and controls for 20 days, 2 runs per day in replicates of 2 (N = 80). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 12 and 13.

Table 12 — Qualitative Analysis of Precision (500 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
375	379	1.7	0.4	375	378	1.8	0.5
500	416	1.7	0.4	500	413	1.5	0.4
625	450	1.9	0.4	625	452	1.3	0.3
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
375	379	2.2	0.6	375	378	2.5	0.7
500	416	2.6	0.6	500	413	2.4	0.6
625	450	2.3	0.5	625	452	2.2	0.5

Table 13 — Semiquantitative Analysis of Precision (500 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
375	377	4.7	1.3	375	371	5.4	1.5
500	501	6.3	1.3	500	489	5.5	1.1
625	655	10.1	1.6	625	668	8.3	1.2
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
375	377	6.4	1.7	375	371	8.6	2.3
500	501	9.4	1.9	500	489	8.1	1.7
625	655	12.0	1.8	625	668	10.7	1.6

1000 ng/mL Cutoff

Accuracy

Qualitative Analysis

One hundred twenty-four (124) specimens were analyzed by the Emit® II Plus Amphetamines Assay and the Emit® II Plus Monoclonal Amphetamine/Methamphetamine Assay (predicate method). Fifty-nine (59) specimens showed positive results by both methods and 61 samples showed negative results by both methods. Three (3) specimens were positive by the Emit® II Plus Amphetamines Assay and negative by the predicate method. One (1) specimen was negative by the Emit® II Plus Amphetamines Assay and positive by the predicate method. Data are summarized in Tables 14 and 15. All specimens positive by both methods were shown to be positive for amphetamines by GC/MS although the ratios of methamphetamine to amphetamine were not consistent with SAMHSA confirmatory guidelines.[1] Fifty (50) of the 61 specimens shown to be negative by both methods were negative for amphetamines by GC/MS.

Table 14 — Accuracy of Qualitative Results

		Predicate Method +	Predicate Method −
Emit® II Plus Amphetamines Assay	+	59	3
	−	1	61

Table 15 — Discrepant Results

Qualitative (mAU/min)	Semiquantitation (ng/mL)		GC/MS (ng/mL)	
Emit® II Plus Amphetamines Assay	Emit® II Plus Amphetamines Assay	Predicate Method	Amphetamine	Methamphetamine
488	1022	905	850	325
481	910	1004	106	995
508	<2000	981	1357	0
495	1200	891	767	508

Cutoff calibrator rate (mAU/min) = 486

One hundred twenty-four (124) specimens were analyzed by the Emit® II Plus Amphetamines Assay and GC/MS (reference method). Fifty-five (55) specimens showed positive results by both methods and 52 samples showed negative results by both methods. Data are summarized in Tables 16 and 17. All positive specimens with both methods were positive by GC/MS according to the SAMHSA requirements by the following criteria: ≥500 ng/mL methamphetamine and ≥200 ng/mL amphetamine or ≥500 ng/mL amphetamine regardless of the methamphetamine concentration.[1]

Table 16 — Accuracy of Qualitative Results

		GC/MS +	GC/MS −
Emit® II Plus Amphetamines Assay	+	55	7
	−	10	52

Table 17 — Discrepant Results

Qualitative (mAU/min)	Semiquantitation (ng/mL)	GC/MS (ng/mL)	
Emit® II Plus Amphetamines Assay	Emit® II Plus Amphetamines Assay	Amphetamine	Methamphetamine
476	847	264	574
484	958	323	808
469	778	1032	161

Qualitative (mAU/min)	Semiquantitation (ng/mL)	GC/MS (ng/mL)	
Emit® II Plus Amphetamines Assay	Emit® II Plus Amphetamines Assay	Amphetamine	Methamphetamine
483	944	525	612
479	889	378	667
476	844	238	727
479	884	269	820
470	788	211	641
466	752	211	644
491	1084	166	883
505	1887	180	1412
485	975	884	327
506	1965	180	1201
504	1753	152	1204
512	<2000	178	1196
512	<2000	182	1254
498	1297	14	979

Cutoff calibrator rate (mAU/min) = 486

Analytical Recovery

Qualitative Results

In qualitative spike analysis, the Emit® II Plus Amphetamines Assay correctly identified the mean rate of spiked specimens containing less than 1000 ng/mL of d-amphetamine or d-methamphetamine as negative, and the mean rate of spiked specimens containing greater than 1000 ng/mL of d-amphetamine or d-methamphetamine as positive.

Semiquantitative Results

Negative human urine was spiked with concentrations of d-amphetamine at levels throughout the semiquantitative range of 150 to 1100 ng/mL. Negative human urine was also spiked with concentrations of d-methamphetamine at levels throughout the semiquantitative range of 200 to 1800 ng/mL. For each known concentration, drug recovery was calculated using the average concentration obtained by the Emit® II Plus Amphetamines Assay. Semiquantitative results are shown in Tables 18 and 19.

Analytical Recovery of Semiquantitative Results

Table 18 — d-Amphetamine Spiked Sample Semiquantitative Analysis (1000 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
150	168	112
225	219	97
300	275	92
450	441	98
550	537	98
750	795	106
900	959	107
1100	1200	109

Table 19 — d-Methamphetamine Spiked Sample Semiquantitative Analysis (1000 ng/mL cutoff)

Nominal Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
200	210	105
250	243	97
330	309	94
500	498	100
750	743	99
1100	1033	94
1500	1443	96
1800	1712	95

Precision

Precision was determined by assaying calibrators and controls for 20 days, 2 runs per day in replicates of 2 (N = 80). Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results are summarized in Tables 20 and 21.

Table 20 — Qualitative Analysis of Precision (1000 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (mAU/min)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
750	471	1.8	0.4	750	475	1.7	0.4
1000	489	1.5	0.3	1000	496	1.9	0.4
1250	501	1.7	0.3	1250	510	2.2	0.4
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
750	471	2.8	0.6	750	475	2.6	0.6
1000	489	3.0	0.6	1000	496	3.1	0.6
1250	501	3.1	0.6	1250	510	4.1	0.8

Table 21 — Semiquantitative Analysis of Precision (1000 ng/mL cutoff)

Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)	Controls/Calibrators (ng/mL)	Mean (ng/mL)	SD	CV (%)
Within-Run Precision							
d-Methamphetamine				**d-Amphetamine**			
750	792	16.1	2.0	750	828	15.9	1.9
1000	982	27.1	2.8	1000	1126	38.7	3.4
1250	1219	43.3	3.6	1250	1670	174.1	10.4
Total Precision							
d-Methamphetamine				**d-Amphetamine**			
750	792	21.6	2.7	750	828	21.6	2.6
1000	982	35.5	3.6	1000	1126	55.7	4.9
1250	1219	65.4	5.4	1250	1670	229.5	13.7

Based on internal testing, the overall reproducibility is estimated to be ≤ 10% CV for samples tested and includes multiple reagent lots, instruments, days, and replicates.[8] Performance of the assay at individual laboratories may vary.

Specificity

The Emit® II Plus Amphetamines Assay detects amphetamine compounds in human urine.

Data found in the following tables are representative of the performance of this assay. However, results may vary among reagent lots.

Table 22 lists the concentrations of amphetamine compounds that produce a result that is approximately equivalent to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL calibrator/control cutoffs. 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 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 22 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 22 — Concentrations of Amphetamines that Produce a Result Approximately Equivalent to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL Amphetamine Cutoffs

	Concentration (ng/mL) Giving a Response Approximately Equivalent to the Cutoff		
Compounds	300 ng/mL Cutoff	500 ng/mL Cutoff	1000 ng/mL Cutoff
d,l-4-Methylamphetamine	4400	10200	16500
d-Amphetamine	300	500	1000
d,l-Methamphetamine	450	700	2100
d,l-Amphetamine	625	1050	2150
l-Methamphetamine	725	1325	3650
l-Amphetamine	3450	3750	11500
1,3 Dimethylpentylamine*	3400	5500	14900
MDA	1100	1700	6500
MDMA	5200	9150	34300
MDEA	4400	6800	27200

*Methylhexanamine

Table 23 lists the concentrations of compounds that produce a result that is approximately equivalent to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL cutoffs. Each concentration represents the reactivity level for the stated compound when it is added to a negative urine specimen. Most of the compounds react at levels much higher than typically found in urine (but which may occasionally occur).[5,9] If a specimen contains more than one compound detected by the assay, lower concentrations than those

listed in Table 23 may combine to produce a rate approximately equivalent to or greater than that of the cutoff calibrator.

Table 23 — Concentrations of Compounds that Produce a Result Approximately Equivalent to the 300 ng/mL, 500 ng/mL, and 1000 ng/mL Amphetamine Cutoffs

	Concentration (µg/mL) Giving a Response Approximately Equivalent to the Cutoff		
Compounds	**300 ng/mL Cutoff**	**500 ng/mL Cutoff**	**1000 ng/mL Cutoff**
4-Chloramphetamine	2.6	4.5	12.2
Benzphetamine*	0.4	0.7	1.0
Bupropion	250	500	2220
Chloroquine	2100	2200	4500
erythro-Dihydrobupropion	20	32	82
Donepezil	6.4	10.2	20.8
l-Ephedrine	400	800	3500
Fenfluramine	25	40	150
Isometheptene	16	29	56
Mephentermine	8	15	60
Methoxyphenamine	90	160	360
Nor-pseudoephedrine	40	70	170
Phenmetrazine	2.3	3.5	13.0
Phentermine	5.8	9.0	25.0
Phenylpropanolamine	700	1000	2000
Propranolol	100	125	500
d,l-Pseudoephedrine	1400	2600	8300
Quinacrine	2500	3800	16500
Tranylcypromine	30	60	200
Tyramine	150	200	600

Benzphetamine metabolizes to amphetamine and methamphetamine.

Note: Selegiline, a prescription medication used in the treatment of Parkinson's disease, metabolizes to l-amphetamine and l-methamphetamine. Therefore, patients taking Selegiline may test positive by amphetamine assays.

Table 24 lists the compounds that produce a negative result by the Emit® II Plus Amphetamines Assay. Specificity testing was performed at the 300, 500, and 1000 ng/mL cutoffs. Positive results for compounds structurally unrelated to amphetamines have not been observed.

Table 24 — Concentrations of Compounds Showing a Negative Response

Compound	300 ng/mL Cutoff (µg/mL)	500 ng/mL Cutoff (µg/mL)	1000 ng/mL Cutoff (µg/mL)
Acetaminophen	1000	1000	1000
α-Acetyl-*N*,*N*-dinormethadol (dinor LAAM)	25	25	25
l-α-Acetylmethadol (LAAM)	25	25	25
N-Acetylprocainamide (NAPA)	400	400	400
Acetylsalicylic Acid	1000	1000	1000
Albuterol	1000	1000	1000
p-Aminobenzoic Acid (PABA)	1000	1000	1000
Amitriptyline	1000	1000	1000
Amoxicillin	1000	1000	1000
Atenolol	1000	1000	1000
Benzoylecgonine	1000	1000	1000
Buprenorphine	1000	1000	1000
Caffeine	1000	1000	1000
Carbamazepine	250	250	250
Carisoprodol	1000	1000	1000
Chlorpheniramine	1000	1000	1000
Chlorpromazine	200	200	200
Cimetidine	1000	1000	1000
Clomipramine	2.5	2.5	2.5
Clonidine	1000	1000	1000
Codeine	500	500	500
l-Cotinine	100	100	100
Cyclobenzaprine	1000	1000	1000
Desipramine	300	500	800
Dextromethorphan	1000	1000	1000
Dextrorphan	280	280	280
Diphenhydramine	1000	1000	1000
Doxepin	1000	1000	1000
Doxylamine	1000	1000	1000
l-Epinephrine	1000	1000	1000
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	1000	1000	1000
Fenoprofen	150	150	150
Fluoxetine	500	500	500
Furosemide	1000	1000	1000
Glutethimide	500	500	500
Haloperidol	500	700	1000
Ibuprofen	1000	1000	1000
Imipramine	750	750	750
Isoxsuprine	300	500	500
Ketamine	100	100	100
Ketoprofen	1000	1000	1000
Ketorolac Tromethamine	1000	1000	1000
Labetalol	750	750	750
Lidocaine	1000	1000	1000
LSD	2.5	2.5	2.5
Meperidine	1000	1000	1000
Mescaline	1000	1500	1500
Methadone	1000	1000	1000
Methaqualone	1500	1500	1500
d,l-Methyldopa	1000	1000	1000
l-Methyldopa	1000	1000	1000
Monoethylglycinexylidide (MEGX)	1000	1000	1000
Morphine	1000	1000	1000
Nalmefene	20	20	20
Naloxone	500	500	500
Naproxen	1000	1000	1000
Nicotinic Acid	500	500	500
Noracetylmethadol (nor LAAM)	25	25	25
11-nor-Δ^9-THC-9-COOH	100	100	100
Nortryptyline	750	750	750
Nylidrin	750	750	750
Ofloxacin	100	100	100
Oxazepam	300	300	300
Phencyclidine	1000	1000	1000
Phenelzine	50	100	100
1-Phenylcyclohexylamine (PCA)	50	50	50
Phenytoin (DPH)	1000	1000	1000
Phthalic Acid	1000	1000	1000
1-Piperidinocyclohexane Carbonitrile (PCC)	50	50	50
Procainamide	1000	1000	1000
Promethazine	1000	1000	1000
Propoxyphene	1000	1000	1000
Ranitidine	1000	1000	1000
Scopolamine	500	500	500
Secobarbital	1000	1000	1000
Thioridazine	100	100	100
Tolmetin Sodium	2000	2000	2000
Tramadol	1000	1000	1000
Trazodone	1000	1000	1000
Trifluoperazine	1000	1000	1000
Trimethobenzamide	500	500	500
Trimethoprim	1000	1000	1000
Verapamil	1000	1000	1000
Zidovudine (AZT)	2000	2000	2000
Zolpidem	100	100	100
Sympathomimetic Amines			
Diethylpropion	1000	1000	1000
d,l-Isoproterenol	1000	1000	1000
Metaproterenol	500	500	500
3,4-Methylenedioxypyrovalerone (MDPV)	100	100	100
4-Methylmethcathione (Mephedrone)	100	100	100
Methylone	100	100	100
Methylphenidate (Ritalin®)	1000	1000	1000

Compound	300 ng/mL Cutoff (µg/mL)	500 ng/mL Cutoff (µg/mL)	1000 ng/mL Cutoff (µg/mL)
Phenethylamine	15	20	20
Phenylephrine	1000	1000	1000
Propylhexedrine	20	30	50
3-OH-Tyramine (dopamine)	300	300	300

Non-Interfering Substances

Each of the following compounds when added to urine containing d-methamphetamine at +/- 25% concentration of the cutoff do not yield a false response relative to the 300, 500, and 1000 ng/mL cutoffs:

Table 25 — Non-Interfering Substances

Compound	Concentration
Acetone	1.0 g/dL
Ascorbic Acid	1.5 g/dL
Bilirubin	2.0 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 of the Emit® II Plus Amphetamines Assay is 100 ng/mL at the 300 ng/mL cutoff and 150 ng/mL at the 500 and 1000 ng/mL cutoffs. This level represents the lowest concentration of d-methamphetamine that can be distinguished from 0 ng/mL with a confidence level of 95%.

11 Bibliography

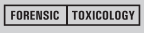
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Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Symbol Title	Source
	Manufacturer	5.1.1[a]
	Use-by date	5.1.4[a]
	Catalog number	5.1.6[a]
	Consult Instructions for Use	5.4.3[a]
	Internet URL address to access the electronic instructions for use	Proprietary
	*In vitro* diagnostic medical device	5.5.1[a]
	Prescription device (US only)	FDA[c]
	CE Marking with Notified Body	EU IVDR[d]
	Temperature limit	5.3.7[a]
	Upper limit of temperature	5.3.6[a]
	Do not re-use	5.4.2[a]
	Recycle	1135[e]
	Biological risks	5.4.1[a]
	Common Units	Proprietary
	Document face up[f]	1952[e]
	Non-sterile	Proprietary

Symbol	Symbol Title	Source
	Authorized representative in the European Community	5.1.2[a]
	Authorized representative in Switzerland	Proprietary
	Batch code	5.1.5[a]
	Contains sufficient for <n> tests	5.5.5[a]
	Version of Instructions for Use	Proprietary
	Revision	Proprietary
	Unique Device Identifier	5.7.10[b]
	CE Marking	EU IVDR[d]
	Keep away from sunlight	5.3.2[a]
	Lower limit of temperature	5.3.5[a]
	Do not freeze	Proprietary
	This way up	0623[e]
	Caution	5.4.4[a]
	International System of Units	Proprietary
YYYY-MM-DD	Date format (year-month-day)	N/A
YYYY-MM	Date format (year-month)	N/A

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Reconstituted volume	Proprietary		Contents	Proprietary
	For forensic/toxicology use only	Proprietary		Level	Proprietary
	Dropper	Proprietary		Cassette	Proprietary
	Not for self-testing	EU IVDR[d]		Not for near-patient testing	EU IVDR[d]

[a] International Standard Organization (ISO). ISO 15223-1 Medical Devices- Symbols to be used with medical device labels, labelling and information to be supplied.

[b] ISO 15223-1:2020-04

[c] Federal Register. Vol. 81, No 115. Wednesday, June 15, 2016. Rules and Regulations: 38911.

[d] IVDR REGULATION (EU) 2017/746

[e] International Standard Organization (ISO). ISO 7000 Graphical symbols for use on equipment.

[f] Indicates Assay-*e*Note

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

Technical Assistance

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

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