SIEMENS Healthineers

Syva®

EMIT® d.a.u.® Amphetamine Class Assay

Shading indicates technical content that differs from the previous version.

10869529_07

Amphetamine Class Assay

1 Intended Use

The Emit® d.a.u.® Amphetamine Class Assay is a homogeneous enzyme immunoassay intended for use in the qualitative analysis of amphetamines in human urine. This assay uses a cutoff level of 300 ng/mL to distinguish positive from negative samples.

The Emit® d.a.u.® Amphetamine Class Assay provides only a preliminary analytical test result. A more specific alternative chemical method[1] 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 and Explanation of the Test

Amphetamine is a general term used to describe several synthetic ephedrine derivatives, the most common of which are D,L-amphetamine, D-amphetamine, and methamphetamine. Amphetamines are readily absorbed after administration and then either deactivated by the liver or excreted unchanged in the urine. The relative importance of these elimination modes depends on urinary pH.[2]

The Emit® d.a.u.® Amphetamine Class Assay uses a cutoff of 300 ng/mL D-amphetamine. The assay detects D,L-amphetamine, D-amphetamine, and methamphetamine in human urine (see Table 4). Other structurally similar phenethylamines can also produce positive results. Compounds giving positive results can be identified by any of several nonimmunochemical analytic procedures, including gas chromatography. The Emit® d.a.u.® Amphetamine Class Assay is an automated assay. Measurements obtained with the amphetamine method are an aid to diagnosis of amphetamines use. A more specific alternative chemical method must be used to obtain a confirmed analytical result.[3]

Methods historically used for detecting amphetamines in biological fluids include thin-layer chromatography, gas chromatography, fluorometry, microcrystallography, enzyme immunoassay, and radioimmunoassay.[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.[1]

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in human urine.[5] The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

REF	Product Description	Quantity/Volume
3C019	**Emit® d.a.u.® Amphetamine Class Assay**	Approximately 300 tests
	Antibody/Substrate Reagent A Sheep antibodies reactive to amphetamine (120 µg/mL),† glucose-6-phosphate (66 mM), nicotinamide adenine dinucleotide (44 mM), Tris buffer, bulking agents, stabilizers, and preservatives	6 mL*
	Enzyme Reagent B Amphetamine labeled with bacterial glucose-6-phosphate dehydrogenase (0.49 U/mL),† Tris buffer, bulking agents, stabilizers, and preservatives	6 mL*
	Emit® Drug Assay Buffer Concentrate When diluted, contains Tris buffer, surfactant, and preservatives	13.3 mL
9A049	**Emit® Calibrator Level 0 (negative)‡**	5 mL*
9A879	**Emit® Calibrator B Level 1 (cutoff)‡**	5 mL*
9A899	**Emit® Calibrator B Level 2 (high)‡** (See Table 1 for drug concentrations)	5 mL*

**** Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.***

† The antibody titer and enzyme conjugate activity may vary from lot to lot.

‡ Required for use with the Emit® d.a.u.® Amphetamine Class Assay. Sold separately.

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

The Emit® calibrators, when reconstituted, contain the concentrations of D-amphetamine listed in Table 1.

Table 1 — D-Amphetamine Concentrations in Emit® Calibrators

Calibrator	Concentration (ng/mL)
Emit® Calibrator Level 0 (negative)	0
Emit® Calibrator B Level 1 (cutoff)*	300
Emit® Calibrator B Level 2 (high)*	2000

**** These calibrators also contain additional drugs of abuse, which do not affect the assay.***

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.

Siemens Healthineers has received authorization for continued use of products containing 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated ('4-tert-OPnEO'; common name 'Triton'):

REACH UK Authorization Numbers 24/06/0 IVD Kit reagents and 24/06/1 IVD wash solutions on diagnostic analyzer systems.

REACH EU Authorization Numbers 23/12/3 IVD Kit reagents and 23/12/4 IVD wash solutions on diagnostic analyzer systems.

Customers in the European Economic Area (EEA) and Northern Ireland using products containing 4-tert-OPnEO shall comply with the following REACH requirements:

1. Per Regulation Number 1907/2006 of the European Parliament, users of this Siemens Healthineers product containing 4-tert-OPnEO must notify European Chemicals Agency (ECHA) by submitting a notification via a REACH IT account.

2. Per Directives 2000/60/EC and 91/271/EEC, users of this Siemens Healthineers product who do not currently collect wastewater shall submit a notification to their competent authority and local wastewater treatment plant indicating the volume of wastewater and the amount of 4-tert-OPnEO that is discharged on an annual basis.

For customer support, contact your local technical support provider or distributor. siemens-healthineers.com

For *in vitro* diagnostic use.

Preparation and Storage of Assay Components

Reagents

To reconstitute Reagents A and B:

- Record the date of reconstitution.
- Remove the metal seal and rubber stopper from the vial.
- Mark the stopper to identify it with the vial.
- Add 6 mL distilled or deionized water.
- Put the stopper back on the vial.
- Swirl the vial until the powder is dissolved.

Let the reconstituted reagents remain at a room temperature of 20–25°C (68–77°F) for at least one hour before use; alternatively, reconstitute the reagents the day before use and store refrigerated (2–8°C, 36–46°F) overnight. Allow reconstituted reagents to reach room temperature before use.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Remove the lid and the seal from the buffer concentrate bottle.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Fill the container to the 200 mL mark with distilled or deionized water.
- Put the cap on the container and invert several times to mix thoroughly.

Table 2 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp	Recon Volume (mL)	Minimum Recon Time & Temp	Stability* Unopened	Stability* Prepared/ Opened
Reagent A	2–8°C (36–46°F)	6	1 h 20–25°C (68–77°F)	Exp date	12 wk
Reagent B	2–8°C (36–46°F)	6	1 h 20–25°C (68–77°F)	Exp date	12 wk
Buffer					
Unopened	2–8°C (36–46°F)		None	Exp date	—
Diluted	20–25°C (68–77°F)	200		—	12 wk
Calibrators†	2–8°C (36–46°F)	5	1 h 20–25°C (68–77°F)	Exp date	12 wk

Stability depends on handling reagents as directed.

†***After reconstitution, always store the calibrators upright.***

Allow all refrigerated components to reach a room temperature of 20–25°C (68–77°F) before use. Do not freeze or expose to temperatures above 32°C (90°F).

5 Specimen Collection and Preparation

- Urine samples may be collected in plastic (ie, polypropylene, polycarbonate, polyethylene) or glass containers. Some plastics adsorb 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.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine samples within the normal urine pH (4.5–8) range do not require prior adjustment of pH.
- Samples outside the normal pH range should be suspected of adulteration.
- Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample.
- Human urine samples should be handled and disposed of as if they were potentially infectious.

6 Procedure

Materials Provided

Emit d.a.u. Amphetamine Class Assay
Reagent A
Reagent B
Drug Assay Buffer Concentrate

Materials Required But Not Provided

Emit Calibrator Level 0 (negative)
Emit Calibrator B Level 1 (cutoff)
Emit Calibrator B Level 2 (high)

Other Items:

Class A volumetric pipette
Distilled or deionized water

Instruments

Siemens Healthineers provides instructions for using this assay on a number of chemistry analyzers. Contact the Technical Assistance Center in the USA or your local Siemens Healthineers 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 Healthineers.

Calibration

Three calibrator levels are available from Siemens Healthineers: the Emit® Calibrator Level 0 (negative), Emit® Calibrator B Level 1 (cutoff), and Emit® Calibrator B Level 2 (high). To analyze your data, use the Emit® Calibrator B Level 1 for qualitative analysis. Validate the calibration by running controls (see Quality Control). Recalibrate as indicated by control results.

Refer to the Emit® Calibrator B instructions for use.

Quality Control

At least once each day of use, analyze two levels of Quality Control (QC) material with known 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

The Emit® Calibrator B Level 1, which contains a concentration of 300 ng/mL D-amphetamine, is used as a reference for distinguishing ''positive'' from ''negative'' samples.

Positive Results

A sample that gives a change in absorbance (ΔA) value equal to or higher than the Calibrator B Level 1 ΔA value is interpreted as positive. The sample contains amphetamines or structurally similar compounds or both.

Negative Results

A sample that gives a change in absorbance (ΔA) value lower than the Calibrator B Level 1 ΔA value is interpreted as negative: Either the sample does not contain amphetamines or amphetamines are present in concentrations below the cutoff level for this assay.

8 Limitations

- The assay is designed for use only with human urine.
- A positive result from the assay indicates the presence of amphetamines, or certain amphetamine-like compounds (see section 10, Specific Performance Characteristics), but does not indicate or measure intoxication.
- Positive results in this assay may also be produced by labetalol; *N,N*-dibenzylethylenediamine (a component of benzathine penicillin); and phenelzine.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

9 Expected Values

When the Emit® d.a.u.® Amphetamine Class Assay is used as a qualitative assay, the amount of drugs and metabolites detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples—positive indicating samples that contain amphetamines or amphetamine-like compounds or both.

10 Specific Performance Characteristics

Data appearing in this section were collected on the Syva® AutoLab Instrument System and on the Syva® ETS® Analyzer.

Accuracy

Accuracy data was obtained using the Emit® d.a.u.® Amphetamine Class Assay with the Emit® d.a.u.® Amphetamine Class calibrators containing D,L-amphetamine.

One hundred seventy-three (173) urine specimens were tested using the Emit® d.a.u.® Amphetamine Class Assay. Eighty-four (84) were positive by the Emit® d.a.u.® assay and 89 were negative by the Emit® d.a.u.® assay. The Emit® d.a.u.® assay results from 171 samples (99%) agreed with results by thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and/or GC/MS. The two remaining samples were negative by the Emit® d.a.u.® assay but positive by the alternative method used.

A separate study was performed to confirm the equivalence of results using either D,L-amphetamine or D-amphetamine calibrators.

A total of 153 clinical urine specimens were tested in two laboratories. Each laboratory tested samples using the Emit® d.a.u.® Amphetamine Class Assay with Emit® calibrators containing 300 ng/mL and 2000 ng/mL D,L-amphetamine and also with Emit® calibrators containing 300 ng/mL and 2000 ng/mL D-amphetamine. The results were identical (100% agreement): 75 samples were positive and 78 were negative using either calibrator set.

In clinical investigations, within-run precision was determined using Emit® calibrators containing 0 ng/mL, 300 ng/mL, and 2000 ng/mL D,L-amphetamine. Results are shown in Table 3.

Table 3 — Within-Run Precision

	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator (0 ng/mL D,L-Amphetamine)	24	288	4.7	1.6
Low Calibrator (300 ng/mL D,L-Amphetamine)	24	385	4.5	1.2
High Calibrator (2000 ng/mL D,L-Amphetamine)	24	520	7.5	1.4

Based on internal testing, the overall reproducibility is estimated to be ≤ 10.0% CV for samples tested and includes multiple reagent lots, instruments, days, and replicates.[6] Performance of the assay at individual laboratories may vary.

Specificity

The Emit® d.a.u.® Amphetamine Class Assay detects major abused amphetamines in urine. Amphetamine-like compounds that can produce positive results include over-the-counter medications such as ephedrine, phenylephrine, pseudoephedrine, and phenylpropanolamine, and prescription medications such as phentermine, mephentermine, phenmetrazine, and diethylpropion. Positive results in this assay may also be produced by labetalol; *N,N*-dibenzylethylenediamine (a component of benzathine penicillin); and phenelzine. Labetalol may be detected at therapeutic concentrations, which are reported to be as high as 39 µg/mL.[7] *N,N*-dibenzylethylenediamine may be detected in the urine of patients receiving therapeutic dosages of benzathine penicillin. Phenelzine may be detected in the urine of patients receiving higher than therapeutic doses of the drug.

Table 4 lists the concentrations of compounds that will produce a positive result.

Table 4 — Amphetamine Class Compounds Detected

Compound	Concentration (ng/mL)
D-Amphetamine	300
D,L-Amphetamine	300
D-Methamphetamine	1000

Table 5 lists amphetamine-like compounds. Concentrations at or above those listed in Table 5 may produce a positive response. The concentrations listed in Table 5 are likely to be found in the urine of individuals taking the listed drugs. Therefore, confirm all positive results by an alternative method.

Table 5 — Concentrations of Amphetamine-Like Compounds Found In Urine

Compound	Concentration (ng/mL)
D,L-Ephedrine	1000
Isometheptene	500
Isoxsuprine	6000
Mephentermine	400
Nylidrin	2000
Phenmetrazine	1000
Phentermine	400
Phenylpropanolamine	1000
Tranylcypromine	750

Table 6 lists the compounds that are not detected by the assay. Positive results for samples containing other compounds structurally unrelated to amphetamines have not been observed.

Table 6 — Compounds Not Detected

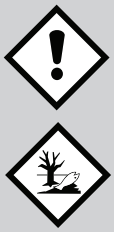
Compound	Concentration Tested (µg/mL)
Benzoylecgonine	1000
Chlorpromazine	12
Dextromethorphan	1000
Diethylpropion	50
Methadone	1000
Morphine	1000
Oxazepam	300
Phencyclidine	1000
Propoxyphene	1000
Secobarbital	1000

11 Risk and Safety

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

H412	Harmful to aquatic life with long lasting effects.
P273, P501	Avoid release to the environment. Dispose of contents and container in accordance with all local, regional, and national regulations
	Contains: Sodium azide; 2-methyl-2H-isothiazol-3-one hydrochloride (Emit d.a.u. Amphetamine Class Antibody/ Substrate Reagent A; Emit Drug Assay Buffer Concentrate)

Contains: 2-methyl-2H-isothiazol-3-one hydrochloride. May produce an allergic reaction. (Emit d.a.u. Amphetamine Class Antibody/Substrate Reagent A; Emit Drug Assay Buffer Concentrate).



H302+H312	**Warning!**
H319	Harmful if swallowed or in contact with skin.
H315	Causes serious eye irritation.
H317	Causes skin irritation.
H411	May cause an allergic skin reaction.
P261, P264,	Toxic to aquatic life with long lasting effects.
P280, P273,	Avoid breathing dust. Wash hands thoroughly after handling.
P302+P352,	Wear protective gloves/protective clothing/eye protection/
P312,	face protection. Avoid release to the environment. IF ON SKIN:
P337+P313,	Wash with plenty of soap and water. Call a POISON CENTER
P333+P313,	or doctor/physician if you feel unwell. If eye irritation persists:
P362+P364,	Get medical advice/attention. If skin irritation or rash occurs:
P391, P501	Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Collect spillage. Dispose of contents and container in accordance with all local, regional, and national regulations.
	Contains: sodium azide; 2-methyl-2H-isothiazol-3-one hydrochloride (Enzyme Reagent B)

CAUTION

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

12 Bibliography

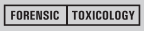
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Definition of Symbols

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 Healthineers:
1-800-227-8994 in the USA
1-800-264-0083 in Canada
Outside the USA and Canada, call your local Siemens Healthineers 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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Siemens Healthcare Diagnostics Inc.
500 GBC Drive
Newark, DE 19714 USA

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