CEDIA® Amphetamine/Ecstasy Assay

microgenics

For In Vitro Diagnostic Use

Catalog No. 100104 (3 x 17 mL Kit)
Catalog No. 100103 (65 mL Kit)
Catalog No. 100040 (495 mL Kit)

Intended Use

The CEDIA® Amphetamines/Ecstasy assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative assay of amphetamines and ecstasy in human urine.

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

Summary and Explanation of the Test

Amphetamines, amphetamine derivatives and ecstasy drugs are classified as sympathomimetic amines with CNS stimulant activity.[2-4] They are psychologically and physiologically addicting, their effects include excitement, alertness, euphoria, decreased appetite, and reduced sense of fatigue.[3, 4] Side effects at low doses include irritability, anxiety, insomnia, blurred vision, increased blood pressure, and heart palpitations.[3, 4] Chronic, high dose users may develop a psychosis that can be indistinguishable from acute schizophrenia.[3, 4]

Amphetamines are rapidly absorbed from the gastrointestinal tract and widely distributed throughout the body.[2, 4] Approximately 70% of a dose is eliminated in urine in the first 24 hours after administration, and depending on urinary pH, about 30% of the dose is excreted unchanged and the remainder as metabolites. Approximately 62% of a methamphetamine dose is eliminated in urine in the first 24 hours after administration, with about 43% of the dose excreted unchanged and the remainder as metabolites, including amphetamine.[2, 4-6] Amphetamines may remain detectable in urine for 3-4 days after administration.[5] MDMA (3,4 - methylenedioxymethamphetamine) is known to be metabolized by N-demethylation to methylenedioxyamphetamine (MDA). The human metabolism of MDA has not been studied; urine concentrations in fatal cases of up to 160 mg/L have been recorded and are indicative of excretion of substantial portions of unchanged drug.[6]

The CEDIA Amphetamines/Ecstasy assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[7] This assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, drug in the sample competes with drug conjugated to one inactive fragment of β-galactosidase for antibody binding site. If drug is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If drug is not present in the sample, antibody binds to drug conjugated to the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme will be formed. The amount of active enzyme formed and resultant absorbance change are proportional to the amount of drug present in the sample.

Reagents

1 ***EA Reconstitution Buffer:*** Contains piperazine-N, N-bis [2-ethanesulfonic acid], buffer salts, stabilizer, and preservative. 4.5 mg/L monoclonal antibody to MDMA.
1a ***EA Reagent:*** Contains 0.156 g/L Enzyme Acceptor, 7.081mg/L monoclonal antibodies to d-amphetamine and 7.081 mg/L mouse monoclonal antibodies reactive to d-methamphetamine, buffer salts, detergent, and preservative.
2 ***ED Reconstitution Buffer:*** Contains piperazine-N, N-bis [2-ethanesulfonic acid] buffer; buffer salts, and preservative.
2a ***ED Reagent:*** Contains 7.12 µg/L Enzyme Donor conjugated to d-amphetamine, 11.3 µg/L Enzyme Donor conjugated to d-methamphetamine, 6.0 µg/L Enzyme Donor conjugated to MDMA, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer, and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100104 and 100103 only. Refer to analyzer specific application sheet for directions on usage.) Empty analyzer bottle for EA/ED solution pour-over (Cat. No. 100103). Empty analyzer bottle for ED solution pour-over (Cat. No. 100040 only).

Additional Materials Required (sold separately):
CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Cutoffs (1000 ng/mL)
CEDIA Multi-Drug Calibrator, Secondary Cutoffs (500 ng/mL)
CEDIA Multi-Drug Intermediate Calibrator
CEDIA Multi-Drug High Calibrator
CEDIA Multi-Drug Control Set (for 1000 ng/mL Cutoff)
CEDIA Specialty Control Set (for 500 ng/mL Cutoff)

Precautions and Warnings

The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Reagent Preparation and Storage

See below for preparation of the solutions for Hitachi analyzers. For all other analyzers, refer to the analyzer specific application sheet. Remove the kit from refrigerated storage immediately prior to preparation of the solutions.

Prepare the solutions in the following order to minimize the risk of possible contamination.

R2 Enzyme donor solution: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at room temperature (15-25°C). Mix again. Record the reconstitution date on the bottle label.

R1 Enzyme acceptor solution: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at room temperature (15-25°C). Mix again. Record the reconstitution date on the bottle label.

Cat. No. 100103 - *Hitachi 717, 911, 912 or 914 analyzer:* Transfer the reconstituted reagents into the corresponding empty R1 and R2 100 mL bottles supplied with kit. ***Hitachi 917 Modular analytics P system:*** Use the reconstituted reagents without transfer of bottles. Discard the empty 100 mL bottles.
Cat. No. 100040 - *Hitachi 747 analyzer/Modular analytics D system:* Use the funnel provided to transfer a portion of the R2 Solution into the appropriately labeled empty R2 Solution bottle provided.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.
NOTE 2: Avoid cross-contamination of reagents by matching reagent stoppers to the proper reagent bottle. The R2 Solution should be yellow-orange in color. A dark red or purple-red color indicates that the reagent has been contaminated and must be discarded.
NOTE 3: The R1 and R2 Solutions must be at the reagent compartment storage temperature of the analyzer before performing the assay. Refer to the analyzer specific application sheet for additional information.
NOTE 4: To ensure reconstituted EA reagent stability, protect from prolonged, continuous exposure to bright light.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.
R1 Solution: 45 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 45 days refrigerated on analyzer or at 2-8°C.

Specimen Collection and Handling

Collect urine samples in clean glass or plastic containers. Centrifuge specimens with high turbidity before testing. Treat human urine as potentially infectious material. Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine samples can affect the test results.

The Mandatory Guidelines for Federal Workplace Drug Testing Programs; Final Guidelines; *Notice* recommends that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.[8]

Assay Procedure

Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instruments parameters are available from Microgenics.

Additional barcode labels are provided for semi-quantitative determination with the 17 mL and 65 mL kits only. To use, over label each bottle with the correct label.

Quality Control and Calibration[9]

Qualitative assay
For **qualitative analysis** of samples, use the Multi-Drug Calibrator, Primary or Secondary Cutoffs (depending on the selected cutoff), to analyze results. See the analyzer specific application sheet.

Semiquantitative assay
500 ng/mL Cutoff Protocol: For **semiquantitative analysis** of samples, use the Multi-Drug Calibrator, Secondary Cutoffs, Primary Cutoffs, Negative Calibrator, and Multi-Drug Intermediate Calibrator.

1000 ng/mL Cutoff Protocol: For **semiquantitative analysis** of samples, use the Mutli-Drug Calibrator, Primary Cutoffs, Negative Calibrator, and the Multi-Drug Intermediate and High Calibrators.

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. It is recommended that two levels of controls be run; one 25% above the cutoff; the other 25% below the cutoff. Recalibrate the test if reagents are changed or if control results are outside of established limits. Base assessment of quality control on the values obtained for the controls, which should fall within specified limits. If any trends or sudden shifts in values are detected, review all operating parameters. Contact Customer Technical Support for further assistance.

Results and Expected Values

Qualitative results
The Multi-Drug Calibrator, Primary or Secondary Cutoffs, (containing 1000 ng/mL and 500 ng/mL d-meth-amphetamine, respectively), is used as a reference in distinguishing between positive and negative samples. Samples producing a response value equal to or greater than the response value of the calibrator are considered positive. Samples producing a response value less than the response value of the calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

Semiquantitative results
500 ng/mL Cutoff Protocol: The Multi-Drug Calibrator, Secondary Cutoffs, used in conjunction with the Negative and the Multi-Drug Calibrator, Primary Cutoffs and the Multi-Drug Intermediate Calibrator, can be used to estimate relative concentration of amphetamines. Refer to the analyzer specific application sheet for detailed information.

1000 ng/mL Cutoff Protocol: The Multi-Drug Calibrator, Primary Cutoffs, used in conjunction with the Negative and the Multi-Drug Intermediate and High Calibrators, can be used to estimate relative concentration of amphetamines. Refer to the analyzer specific application sheet for detailed information.

Care should be taken when reporting concentration results since there are many other factors that may influence a urine test result such as fluid intake and other biological factors.

Limitations

1. A positive test result indicates the presence of amphetamines; it does not indicate or measure intoxication.
2. Other substances and/or factors not listed may interfere with the test and cause false results (e.g., technical or procedural errors).

Specific Performance Characteristics

Typical performance results obtained on the Hitachi 717 analyzer are shown below.[10] The results obtained in your laboratory may differ from these data.

Precision

Measured precision studies, using packaged reagents, calibrators, and controls yielded the following results with a Hitachi 717 analyzer using NCCLS modified replication experiment guidelines.

Qualitative (mA/min):

	Within-run Precision				Total Precision			
ng/mL	500*	750**	1000**	1250**	500*	750**	1000**	1250**
n	120	120	120	120	120	120	120	120
$\bar{x}$	353.0	336.1	360.0	385.9	353.0	336.1	360.0	385.9
SD	3.0	4.2	4.1	5.2	5.7	6.5	7.0	7.6
%CV	0.9	1.3	1.1	1.4	1.6	1.9	2.0	2.0

*Determined using the 500 ng/mL cutoff protocol
** Determined using the 1000 ng/mL cutoff protocol

Semiquantitative (ng/mL):

	Within-run Precision				Total Precision			
ng/mL	500*	750**	1000**	1250**	500*	750**	1000**	1250**
n	120	120	120	120	120	120	120	120
$\bar{x}$	496.5	808.5	1057.6	1403.6	496.5	808.6	1057.6	1403.6
SD	27.0	29.9	51.2	68.7	38.6	52.6	77.3	115.1
%CV	5.4	3.7	4.8	4.9	7.8	6.5	7.3	8.2

*Determined using the 500 ng/mL cutoff protocol
** Determined using the 1000 ng/mL cutoff protocol

Accuracy

Urine samples were assayed with the CEDIA Amphetamines/Ecstasy assay on the Hitachi 717 analyzer using GC/MS and commercially available CEDIA DAU Amphetamines assay as references. Results were as follows:

A. 500 ng/mL Cutoff

GC/MS \ CEDIA (Amphetamine/Ecstasy)	+	-
+	158	1
-	8	17

B. 1000 ng/mL Cutoff

CEDIA (Amphetamine/Ecstasy) \ CEDIA (Amphetamine)	+	-
+	144	18
-	0	87

	GC/MS (ng/mL)*		CEDIA Assay (ng/mL)*			% Agreement
Ranges	Min	Max	Positive	Negative	n	%
0	0	0	0	5	5	100%
0 to -50% CO*	40	207	1	5	6	83%
-50% CO to -25% CO	277	351	3	2	5	40%
-25% CO to CO	392	495	4	5	9	56%
Assay CO	500	500	2	0	2	100%
CO to +25% CO	502	575	7	1	8	88%
+25% CO to +50% CO	635	693	3	0	3	100%
>50% CO	>750	---	146	0	146	100%

*CO=Cutoff (500 ng/mL as Cutoff)

Specificity

The following compounds when tested with the CEDIA Amphetamines/Ecstasy assay, 1000 ng/mL cutoff protocol, yielded the following percent cross-reactivity results:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
d-Amphetamine	1000	104
l-Amphetamine	40,000	1.0
d,l-Amphetamine	1,250	88
d,l-Methamphetamine	1,000	77
l-Methamphetamine	8,000	18
3,4-Methylenedioxy-amphetamine (MDA)	1000	116
3,4-Methylenedioxy-methamphetamine (MDMA)	500	196
3,4-Methylenedioxy-ethylamphetamine (MDEA)	300	172
N-Methylbenzodioxa-zolylbutanamine (MBDB)	900	121
Benzodioxazolybutanamine (BDB)	1000	76
Phentermine	25,000	3.3
d,l-Phenylpropanolamine	500,000	0.3
d-Pseudoephedrine	160,000	0.9
l-Ephedrine	250,000	0.5
p-Methoxyamphetamine (PMA)	2000	24
p-Methoxymethamphetamine(PMMA)	500	100

Structurally unrelated compounds were tested with the CEDIA Amphetamines/Ecstasy assay, 500 ng/mL cutoff protocol, and gave a negative response when tested at the concentrations listed.

Compound	ng/mL	Compound	ng/mL
Acetaminophen	1,000,000	Levothyroxine	100,000
Acetylsalicylic acid	1,000,000	Methadone	1,000,000
Amoxicillin	1,000,000	Morphine	1,000,000
Benzoylecgonine	1,000,000	Nifedipine	50,000
Captopril	1,000,000	Phencyclidine	1,000,000
Chlordiazepoxide	250,000	Phenobarbital	1,000,000
Cimetidine	500,000	d-Propoxyphene	1,000,000
Codeine	1,000,000	Ranitidine	250,000
Diazepam	100,000	Salicyluric acid	1,000,000
Digoxin	1,000,000	Secobarbital	1,000,000
Enalapril	1,000,000	11-nor-Δ^9-THC-COOH	10,000
Fluoxetine	500,000	Verapamil	1,000,000
Ibuprofen	1,000,000	Tolmetin	500,000

No interference was observed from the folowing substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Amphetamines/Ecstasy assay:

Substance	Concentration	Substance	Concentration
Acetone	≤ 1.0 g/dL	Hemoglobin	≤ 0.3 g/dL
Ascorbic acid	≤ 1.5 g/dL	Human serum albumin	≤ 0.5 g/dL
Creatinine	≤ 0.5 g/dL	Oxalic acid	≤ 0.1 g/dL
Ethanol	≤ 1.0 g/dL	Riboflavin	≤ 7.5 mg/dL
Galactose	≤ 10 mg/dL	Sodium Chloride	≤ 6.0 g/dL
γ-globulin	≤ 0.5 g/dL	Urea	≤ 2.0 g/dL
Glucose	≤ 1.5 g/dL		

Sensitivity

For the Qualitative application, the limit of detection (LOD) was 35 ng/mL and 75 ng/mL for the 500 ng/mL and 1000 ng/mL cutoff protocols, respectively.

For the Semiquantitative application, the LOD was 41 ng/mL and 99 ng/mL for the 500 ng/mL and 1000 ng/mL cutoff protocols, respectively.

References

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5. Busto U, Bendayan R, Sellers EM. *Clinical pharmacokinetics of non opiate abused drugs*. 1989, **16**: 1-26.
6. Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*. 4th ed. Foster City, Calif.: Chemical Toxicolog Instititute; 1995
7. Henderson DR, Friedman SB, Harris JD. et al. CEDIA®, a new homogeneous immunoassay system. *Clin Chem*. 1986; **32**:1637-1641
8. *Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program:* Revised Mandatory Guidelines. *Federal Register*. 1994;**110** (June 9): 11983 (Revised Guidelines expected in 2002.)
9. Data on traceability are on file at Microgenics Corporation.
10. Data on file at Microgenics Corporation.

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