CEDIA® Heroin Metabolite (6-AM) Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100107 (3 x 17 mL)
10015213 (3 x 17 mL Indiko Kit)
100108 (65 mL Kit)
100186 (495 mL Kit)

Intended Use

The CEDIA® Heroin Metabolite (6-Acetylmorphine, or 6-AM) Assay is a homogeneous enzyme immunoassay for the in vitro qualitative and semiquantitative determination of heroin metabolite (6-AM) in human urine on automated clinical chemistry analyzers. Measurements are used as an aid in the detection of heroin use or overdose.

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 judgement should be applied to any drug of abuse test result particularly when preliminary positive results are used.

Summary and Explanation of the Test

Heroin (3,6-diacetylmorphine) is produced by chemical modification of morphine, a naturally-occurring alkaloid found in the unripe pods of the opium poppy Papaver somniferum.[2,3] Heroin is highly addictive, and is currently a Schedule I substance (no currently accepted medical use). Heroin is the most abused opiate drug,[4,5] and its use is associated with a wide variety of health problems. Heroin is administered by intravenous or subcutaneous injection or by nasal insufflation.[3] It is rapidly metabolized (half-life of 9 minutes) to 6-AM by esterases in the blood, and then to morphine by hydrolysis in the liver.

The presence of 6-AM in urine is regarded as a specific marker for the illicit use of heroin.[6-8] 6-AM cannot be formed by acetylation of morphine in the body; thus the presence of 6-AM cannot be caused by injection of legal opiate analgesics or large quantities of poppy seeds. For this reason, the Department of Health and Human Services (DHHS) introduced revised guidelines for opiate testing which required testing of all opiate-positive urine specimens for 6-AM for confirmation of heroin abuse.[9] The half-life of 6-AM is approximately 35 minutes. The time for which the measurement of 6-AM is diagnostic of heroin use is dependent on the amount of heroin taken. It is likely that even for higher doses of heroin the detection time is limited to 24 hours after use.[6]

The CEDIA Heroin Metabolite Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[10] This assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments, termed Enzyme Acceptor (EA) and Enzyme Donor (ED) 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 CEDIA Heroin Metabolite Assay, the sample competes with 6-AM conjugated to ED for antibody binding sites. If 6-AM is present in the sample, it binds to antibody, leaving the ED-6-AM conjugate free to reassociate with EA to form active β-galactosidase. If no 6-AM is present in the sample, antibody binds to the ED-6-AM conjugate, inhibiting the reassociation of inactive β-galactosidase fragments, and thus reducing the amount of active enzyme formed. The amount of active enzyme formed and resultant absorbance change are proportional to the amount of 6-AM present in the sample.

Reagents

1 **EA Reconstitution Buffer:** Contains 0.32 mg/L mouse monoclonal antibodies to 6-Acetylmorphine, buffer salts, detergent and preservative.
1a **EA Reagent:** Contains 0.171 g/L enzyme acceptor, buffer salts, detergent and preservative.
2 **ED Reconstitution Buffer:** Contains buffer salts and preservative.
2a **ED Reagent:** Contains 16.2 µg/L enzyme donor conjugated to 6-Acetylmorphine, 1.67 g/L chlorophenol red-β-D galactopyranoside, stabilizer and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100107 and 100108. Refer to analyzer specific application sheet for directions on usage.) Empty analyzer bottles for EA/ED solutions pour-over (Cat. No. 100108). Empty analyzer bottles for ED solutions pour-over (Cat. No. 1000186 only).

Additional Materials Required (sold separately):

1557416	CEDIA Negative Calibrator, 5 mL
1661388	CEDIA Negative Calibrator, 10 mL
100031	CEDIA Heroin Metabolite (6-AM) Cutoff Calibrator, 5 mL
100034	CEDIA Heroin Metabolite (6-AM) High Calibrator, 5 mL
100202	MGC Select Control Set, 3 x 5 mL

⚠ Precautions and Warnings

1. This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. Do not use the reagents beyond their expiration dates.

DANGER: Powder reagent contains ≤55% w/w bovine serum albumin (BSA), and ≤1% w/w Sodium azide. Liquid reagent contains ≤0.5% bovine serum, ≤0.15% Sodium azide and <0.1% Drug-specific antibody (Mouse).
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust/mist/vapors/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms; Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/ container to location in accordance with local/regional/national/international regulations.

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. 100108-Hitachi 717, 911, 912 or 914 analyzer: Transfer the reconstituted reagents into the corresponding empty R1 and R2 100 mL bottles supplied with the kit. **Hitachi 917/Modular analytics P system:** Use the reconstituted reagents without transfer of bottles. Discard the empty 100 mL bottles.
Cat. No. 100186-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 sheet for additional information.
NOTE 4: To ensure reconstituted EA solution 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: 60 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 60 days refrigerated on analyzer or at 2-8°C.

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Specimens kept at room temperature that do not receive initial test within 7 days[11] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for two months.[12] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[12, 13]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[9]

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. An effort should be made to keep pipetted samples free of gross debris. It is recommended that grossly turbid specimens be centrifuged before analysis. Frozen samples should be thawed and mixed prior to analysis. Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing.

Handle all urine specimens as if they were potentially infectious.

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 instrument parameters are available from Microgenics, a part of Thermo Fisher Scientific.

Quality Control and Calibration[14]

Qualitative analysis

For analysis of samples, use the CEDIA Heroin Metabolite (6-AM) Cutoff Calibrator. For all other analyzers, see the analyzer specific application sheet.

Semiquantitative analysis

For semiquantitative analysis of samples, use the CEDIA Negative Calibrator and the CEDIA Heroin Metabolite Cutoff Calibrator to analyze results. For all other analyzers, see the analyzer specific application sheet. Recalibrate the test if reagents are changed or if control results are outside of established limits.

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 controls be run; a positive control and a negative control. 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. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

Qualitative results

The CEDIA Heroin Metabolite (6-AM) Cutoff calibrator (10 ng/mL) is used as a reference in distinguishing between positive and negative samples. Samples producing a response value that is equal to or greater than the response value of the cutoff calibrator are considered positive. Samples producing a response value less than the response value of the cutoff calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

Semiquantitative results

The CEDIA Heroin Metabolite (6-AM) Cutoff Calibrator used in conjunction with the CEDIA DAU Negative Calibrator, can be used to estimate relative concentration of 6-Acetylmorphine. 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.

Limitations

1. A positive test result indicates the presence of 6-AM; it does not indicate or measure intoxication.
2. There is a possibility that other substances and/or factors (e.g., technical or procedural errors) not may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance results obtained on the Hitachi 717 analyzer are shown below.[15] Results obtained in your laboratory may differ from these data.

Precision

Measured precision studies, using packaged reagents, calibrators, and controls yielded the following results in mA/min with a Hitachi 717 analyzer following NCCLS modified replication experiment (6 replicates twice daily for 10 days).

Hitachi 717 Qualitative

Using the 10 ng/mL cutoff calibrator	Within-run Precision		Total Precision	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Low Control (7.5 ng/mL)	422 ± 2.2	0.5	405 ± 5.9	1.5
Cutoff	455 ± 1.9	0.4	434 ± 7.4	1.7
High Control (12.5 ng/mL)	486 ± 3.1	0.6	454 ± 12.2	2.7

Hitachi 717 Semiquantitative

Using the 10 ng/mL cutoff calibrator	Within-run Precision		Total Precision	
	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Low Control (7.5 ng/mL)	7.6 ± 0.41	5.32	7.6 ± 0.49	6.41
Cutoff	9.9 ± 0.42	4.27	9.9 ± 0.53	5.32
High Control (12.5 ng/mL)	12.0 ± 0.43	3.55	12.0 ± 0.54	4.49

Accuracy

A total of 236 urine samples were assayed with the CEDIA Heroin Metabolite (6-AM) Assay on the Hitachi 717 analyzer using GC/MS as reference. Results were as follows:

		Hitachi CEDIA 6-AM Assay +	Hitachi CEDIA 6-AM Assay -
GC/MS	+	189	1*
	-	0	46

*Sample contained 10.7 ng/mL 6-Acetylmorphine by GC/MS.

Specificity

The following parent compounds, metabolites and structurally related compounds, when tested with the CEDIA Heroin Metabolite (6-AM) Assay, yielded negative results against the cutoff calibrator (10 ng/mL):

Compound	Concentration Tested (ng/mL)
Codeine	500,000
Dextromethorphan	100,000
Dihydrocodeine	500,000
Heroin HCl	80
Hydrocodone	300,000
Hydromorphone	10,000
Imipramine	200,000
Levorphanol	10,000
Meperidine	800,000
Morphine	9,000
Morphine-3-Glucuronide	600,000
Morphine-6-Glucuronide	600,000
Nalorphine	7,000
Naloxone	300,000
Naltrexone	300,000
Norcodeine	600,000
Normorphine	30,000
Oxycodone	400,000
Oxymorphone	80,000

Structurally unrelated compounds were tested with the CEDIA Heroin Metabolite (6-AM) Assay and gave a negative response when tested at the concentrations listed below.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
10, 11 Dihydrocarbamazepine	85,000	Haloperidol	100,000
11-nor-Δ^9-THC-COOH	10,000	Hydroxyzine	500,000
Acetaminophen	500,000	Ibuprofen	500,000
Acetylsalicylic Acid	500,000	Levothyroxine	50,000
Amitriptyline	500,000	Methadone	500,000
Amoxicillin	500,000	Methamphentamine	500,000

Table Continued

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Benzotropine Methane Sulfonate	500,000	Nifedipine	500,000
Benzoylecgonine	100,000	Nordiazepam	100,000
Brompheniramine	75,000	Oxazepam	250,000
Caffeine	500,000	Perphenazine	150,000
Captopril	500,000	Phencyclidine	30,000
Chlordiazepoxide	100,000	Phenobarbital	500,000
Chlorpromazine	10,000	Procyclidine	800,000
Cimetidine	500,000	Propoxyphene	100,000
Desipramine	500,000	Protriptyline	200,000
Diazepam	100,000	Ranitidine	500,000
Digoxin	100,000	Salicyluric Acid	500,000
Diphenhydramine	50,000	Secobarbital	500,000
Doxepine HCl	100,000	Triprolidine	50,000
Enalapril	500,000	Verapamil	500,000
Fluoxetine	500,000		

Interference

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Heroin Metabolite (6-AM) assay:

Substance	Concentration	Substance	Concentration
Acetone	≤ 1.0 g/dL	Hemoglobin	≤ 0.3 mg/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	≤ 1.0 g/dL		

References

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11. Zaitsu K, Miki A, Katagi M, Tsuchihashi H. Long-term stability of various drugs and metabolites in urine, and preventive measures against their decomposition with special attention to filtration sterilization. *Forensic Science Intl* 174 (2008) 189-196.
12. Gonzales E, Ng G, Pesce A, West C, West R, Mikel C, Llaatyshev, S, Almazan P. Stability of pain-realted medications, metabolites and illicit substances in urine. *Clinica Chimca Acta* 416: (2013) 30-35.
13. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
14. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
15. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

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10006723-10-EN
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