CEDIA™ Cocaine Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10016413 (3 x 17 mL Indiko Kit)
100086 (3 x 17 mL Kit)
100095 (65 mL Kit)
1661230 (495 mL Kit)

Intended Use
The CEDIA™ Cocaine assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative assay of cocaine metabolites in human urine.

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
Cocaine (benzoylmethylecgonine), is derived from the plant species Erythroxylon coca, which is widely grown in South America.[2-4]

Cocaine is popularly abused in the US.[2,3,5] Cocaine abuse can produce euphoria, arousal, garrulousness, alertness, anxiety, insomnia, hyperactivity, paranoia, severe psychosis, and even suicide.[2,5,6]

Cocaine is rapidly metabolized, with less than 5% excreted unchanged in the urine.[3,5,6] The two major metabolites, which result from enzymatic and nonenzymatic hydrolysis, are benzoylecgonine and ecgonine methyl ester.[3,5-8] The metabolites may be detectable in urine for up to 3 weeks after long term, heavy use of cocaine.[9,10]

The CEDIA Cocaine assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[11] 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 on 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], 0.54 µg/mL mouse monoclonal antibodies to benzoylecgonine, buffer salts, stabilizer, and preservative.
1a EA Reagent: Contains 0.171 g/L Enzyme acceptor, buffer salts, detergent and preservative.
2 ED Reconstitution Buffer: Contains Piperazine-N, N-bis [2-ethanesulfonicacid]; buffer salts, and preservative.
2a ED Reagent: Contains 15.38 µg/L Enzyme donor conjugated to benzoylec-gonine, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer, and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100086 and 100095 only. Refer to analyzer specific application sheet for directions on usage). Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100095). Empty analyzer bottle for ED solution pour-over (Cat. No. 1661230 only).

Additional MaterialsRequired (sold separately):
CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Cutoffs or Primary Clinical Cutoff, (300 ng/mL)
CEDIA Multi-Drug Calibrator, Secondary Cutoffs, (150 ng/mL)
CEDIA Multi-Drug Calibrator, Optional Cutoffs, (200 ng/mL)
CEDIA Multi-Drug Intermediate Calibrator,
CEDIA Multi-Drug High Calibrator,
MGC Primary DAU Control Set, (300 ng/mL)
MGC Clinical DAU Control Set, (300 ng/mL)
MGC Select DAU Control Set, (150 ng/mL)
MGC Specialty DAU Control Set, (150 ng/mL)

⚠ Precautions and Warnings
DANGER:
Powder Reagent contains ≤56% w/w bovine serum albumin (BSA), and ≤2% w/w sodium azide. Liquid Reagent contains ≤1.0% bovine serum, ≤0.3% 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.

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. 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. 100095-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. 1661230-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 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 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[12] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for two months.[13] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[13, 14]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[15]

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 enzymtic 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.

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[16]

Qualitative analysis

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

Semiquantitative analysis

For **semiquantitative analysis** of samples, use the Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs (depending on the selected cutoff) in conjunction with the Negative Calibrator, and the Multi-Drug Intermediate and High Calibrators to analyze results.

Good laboratory practice suggests that controls be run 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. Use the CEDIA Multi-Drug Control Set, Clinical Control Set or Optional Control Set (300 cutoff) or Specialty Control Set, (150 cutoff) for quality control. Recalibrate the test if reagents are changed or if control results are outside of established limits. Each laboratory should establish its own control frequency. 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 Multi-Drug Calibrator, Primary or Secondary Cutoffs, (depending on selected cutoff), 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 value of the calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

Semiquantitative results

The Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs, used in conjunction with the Negative and the Multi-Drug Intermediate and High Calibrators, can be used to estimate relative concentration of cocaine metabolites. 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 cocaine metabolites; it does not indicate or measure intoxication.
2. Other substances and/or factors not listed may interfere with the test and cause false results (eg. technical or procedural errors).

Specific Performance Characteristics

Typical performance results obtained on the Hitachi 717 analyzer are shown below.[17] The results obtained in your laboratory may differ from these data.

Precision

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

	Within-run precision				Total precision			
ng/mL	150	225	300	375	150	225	300	375
n	120	120	120	120	120	120	120	120
$\bar{x}$	292.6	333.4	363.6	387.3	292.6	333.4	363.6	387.3
SD	4.15	3.36	3.32	3.21	13.6	8.91	9.69	10.47
%CV	1.4	1.0	0.9	0.8	4.7	2.7	2.7	2.7

Accuracy

Five hundred and ninety urine samples were assayed with the CEDIA Cocaine assay on the Hitachi 717 analyzer using a commercial EIA method for cocaine metabolites as reference. Results were as follows:

A. 150 ng/mL Cutoff

EIA \ CEDIA	+	-
+	94	2*
-	0	494

B. 300 ng/mL Cutoff

EIA \ CEDIA	+	-
+	87	5†
-	0	498

* Both samples were tested by GC/MS and were found to contain 22 and 94 ng/mL benzoylecgonine respectively.

† All 5 samples were tested by GC/MS. Four samples were found to contain 22-10 ng/mL benzoylecgonine. The fifth sample was found to contain 169 ng/mL benzoylecgonine.

Specificity

The following parent compounds and metabolites, when tested with the CEDIA Cocaine assay, 300 ng/mL cutoff protocol, yielded the following percent cross-reactivity results:

Compound	Concentration Tested (ng/mL)	% Cross Reactivity
Benzoylecgonine	300	100
Cocaethylene	312	57
Cocaine	315	54
Ecgonine	10,000	1.1
Ecgonine methyl ester	10,000	< 0.1

Structurally unrelated compounds were tested with the CEDIA Cocaine assay, 300 ng/mL cutoff protocol, and gave a negative response when tested at the concentrations listed below.

Compound	ng/mL	Compound	ng/mL
Acetaminophen	500,000	Levothyroxine (T4)	50,000
Acetylsalicylic acid	500,000	Methadone	500,000
Amoxicillin	100,000	Methamphetamine	500,000
Amphetamine	500,000	Morphine	100,000
Captopril	500,000	Nifedipine	500,000
Chlordiazepoxide	100,000	Phencyclidine	500,000
Cimetidine	500,000	Phenobarbital	500,000
Codeine	500,000	Propoxyphene	500,000
Diazepam	500,000	Ranitidine	500,000
Digoxin	100,000	Salicyluric acid	500,000
Enalapril	500,000	Secobarbital	500,000
Fluoxetine	500,000	11-nor-Δ^9-THC-COOH	10,000
Ibuprofen	500,000	Verapamil	500,000

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Cocaine assay:

Substance	Concentration	Substance	Concentration
Acetone	≤ 1.0 g/dL	Hemoglobin	≤ 0.3 g/dL
Ascorbic acid	≤ 0.15 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 6 ng/mL and 13 ng/mL for the 150 ng/mL and 300 ng/mL cutoff protocols, respectively.

For the Semiquantitative application, the LOD was 13.2 ng/mL and 19.5 ng/mL for the 150 ng/mL and 300 ng/mL cutoff protocols, respectively.

References

1. Hawks RL. Analytical methodology. In: Hawks, RL,Chiang, CN, eds. Urine Testing for Drugs of Abuse. NIDA Research Monograph 1986, 73: 30-41.
2. Gawin FH, Ellinwood EH Jr. Cocaine and other stimulants: Actions, abuse, and treatment. N. Engl. J. Med. 1988, 318: 1173-1182.
3. Jatlow, Pl. Drugs of abuse profile: Cocaine. Clin. Chem. 1987, 33 (suppl): 66B-71B.
4. Bouknight LG, Bouknight RR. Cocaine - A particularly addictive drug. Postgrad. Med.1988; 83: 115-124,131.
5. Benowitz, NL, Clinical pharmacology and toxicology of cocaine. Pharmacol. & Toxicol.1993; 72: 3-12.
6. Jones RT. The pharmacology of cocaine. In: Grabowski, J. ed. Cocaine: Pharmacology, Effects and Treatment of Abuse. NIDA Research Monograph. 1984; 50: 34-53.
7. Ambre J. Urinary excretion of cocaine and metabolites in humans: A kinetic analysis of published data. J. Anal. Toxicol. 1985; 9: 241-245.
8. Ambre J, Ruo TI, Nelson J, Belknap S. Urinary excretion of cocaine, benzoylecgonine and ecgonine methyl ester in humans. J. Anal. Toxicol. 1988; 12: 301-306.
9. Weiss RD. Gawin, FH. Protracted elimination of cocaine metabolites in long-term, high-dose cocaine abusers. Am. J. Med. 1988, 85: 879-880.
10. Burke WM, et al. Prolonged presence of metabolite in urine after compulsive cocaine use. J. Clin. Psychiatry. 1990; 51:145-148.
11. Henderson DR, Friedman SB, Harris JD et al. CEDIA, a new homogeneous immunoassay system. Clin. Chem. 1986; 32: 1637-1641.
12. Kiszka M, Buszewicz G, Mądro R. This article is based on a presentation given at the 11th Meeting of the Polish Society of Forensic Medicine and Criminology, £ódŸ, 1998.
13. Gonzales E, Ng G, Pesce A, West C, West R, Mikel C, Llaatyshev S, Almazan P. Stability of pain-related medications, metabolites and illicit substances in urine. *Clinica Chimca Acta* 416: (2013) 30-35.
14. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
15. *Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program: Final Guidelines; Federal Register,* Substance Abuse and Mental Health Administration (SAMHSA), (1994) 110 (June 9):11983.
16. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
17. Data on file at Microgenics Corporation., a part of Thermo Fisher Scientific.

Glossary:

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

Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
B·R·A·H·M·S GmbH
Neuendorfstrasse 25
16761 Hennigsdorf, Germany

For insert updates go to:
www.thermofisher.com/diagnostics

Other countries:

Please contact your local Thermo Fisher Scientific representative.

10006475-7-EN
2019 06

thermo scientific