

CEDIA™ Opiate Assay

IVD For In Vitro Diagnostic Use

Rx Only

REF 10016429 (3 x 17 mL Indiko Kit)
100089 (3 x 17 mL Kit)
100098 (65 mL Kit)
1661248 (495 mL Kit)

Intended Use

The CEDIA™ Opiate Assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative assay of opiates 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 judgement should be applied to any drug of abuse test result particularly when preliminary positive results are used.

Summary and Explanation of the Test

Opium is obtained from the unripe pods of the opium poppy *Papaver somniferum*.^{2,3} Morphine and codeine are naturally occurring alkaloids of opium.^{2,4} Both have widely accepted medical uses, principally as analgesics; however, both drugs are sometimes abused.^{2,5} Heroin is a compound synthesized from morphine and is the most commonly abused opiate.⁷

Opiates (morphine, codeine and heroin) are rapidly metabolized by the body, and the main site of metabolism is the liver.^{4,8} Morphine is excreted in urine as conjugated morphine, free morphine and other trace metabolites.^{3,4,8} Codeine is excreted in urine as free and conjugated codeine and free and conjugated morphine.^{2,5} After codeine administration, total codeine may be eliminated faster than total morphine so that some urine specimens of codeine users may show only the presence of total morphine or a ratio of total morphine to total codeine of greater than one.^{3,5,7} Heroin is rapidly metabolized in whole blood to 6-monoacetylmorphine, which is then hydrolyzed to conjugated morphine in the liver.^{3,6} It is excreted in urine principally as conjugated morphine, but also in small amounts as free morphine and 6-monoacetylmorphine.^{3,6} Depending on the dose and the sensitivity of the analytical method, total morphine may be detected in urine up to 72 hours after last administration of morphine, codeine, or heroin.^{3,5,7,8}

The CEDIA Opiate assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.⁹ 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

- EA Reconstitution Buffer:** Contains citrate buffer, 3 μ g/mL mouse monoclonal antibodies to opiates, buffer salts, stabilizer, and preservative.
- 1a EA Reagent:** Contains 0.171 g/L Enzyme Acceptor, buffer salts, detergent, and preservative.
- ED Reconstitution Buffer:** Contains phosphate buffer, buffer salts and preservative.
- 2a Enzyme Donor Reagent:** Contains 23.3 μ g/L Enzyme Donor conjugated to morphine, 1.67 g/L chlorophenol red- β -D-galactopyranoside, stabilizer, and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100089 and 100098 only). Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100098). Empty analyzer bottle for ED solution pour-over (Cat. No. 1661248 only).

Additional Materials Required (sold separately):

CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Clinical Cutoffs,
Secondary Cutoffs, or Optional Cutoffs, (300 ng/mL)
CEDIA Multi-Drug Intermediate Calibrator
CEDIA Multi-Drug High Calibrator
CEDIA Multi-Drug Clinical Control Set, Speciality Control Set or Optional Control Set

⚠ Precautions and Warnings

DANGER: Powder reagent contains $\leq 56\%$ w/w bovine serum albumin (BSA) and $\leq 2\%$ w/w sodium azide. Liquid reagent contains $\leq 1.0\%$ bovine serum, $\leq 0.3\%$ sodium azide and $\leq 0.1\%$ 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

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.

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 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.

Specimens kept at room temperature that do not receive initial test within 7 days¹⁰ of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for two months.¹¹ For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.^{11, 12}

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.¹³

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.

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 Calibration¹⁴

Qualitative analysis

For qualitative analysis of samples, use the Multi-Drug Calibrator, Primary Clinical Cutoff, Optional Cutoffs or Secondary Cutoffs, to analyze results. See the analyzer specific application sheet.

Semiquantitative analysis

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

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. Use the CEDIA Multi-Drug Clinical Control Set, Specialty Control Set or Optional Control Set 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 Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs, (each containing 300 ng/mL morphine), 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 cutoff calibrator are considered positive. Samples producing a response value less than the value of the cutoff calibrator are considered negative. Refer to analyzer specific application sheet for additional information.

Semiquantitative results

The Multi-Drug Calibrator, 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 opiates.

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 opiates; it does not indicate or measure intoxication.
2. Poppy seeds can contain opiates, and ingestion of products containing poppy seeds can cause a positive test result.^{15,16}
3. Other substances and/or factors not listed may interfere with the test and cause false results (e.g., technical or procedural errors).
4. When the semiquantitative procedure is performed, results of the CEDIA Opiate assay yield only approximate cumulative concentrations of the drug being tested.

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. For additional analyzer specific performance results, refer to the analyzer specific application sheet.

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.

ng/mL	Within-run Precision			Total Precision		
	225	300	375	225	300	375
n	120	120	120	120	120	120
\bar{x}	269.8	316.8	361.4	269.8	316.8	361.4
SD	4.23	4.54	4.84	11.38	15.04	15.27
%CV	1.6	1.4	1.3	4.2	4.8	4.2

Accuracy

Six hundred urine samples were assayed with the CEDIA Opiate assay on the Hitachi 717 analyzer and by the Syva Emit[®] II Opiate Assay (reference method). Results were as follows:

		CEDIA	
		+	-
Emit [®] II	+	100	0
	-	3*	497

Sensitivity 100%
Specificity 99.4%

*2 of the 3 samples were tested by GC/MS and were found to contain 227 and 241 ng/mL total morphine, respectively. The third sample was tested by the Emit[®] II semiquantitative protocol and gave a concentration of 196 ng/mL. The sample was tested by GC/MS and was found to contain high concentrations of imipramine and its metabolites.

Specificity

The following parent compounds and metabolites, when tested with the CEDIA Opiate assay, yielded the following percent cross-reactivity results:

Compound	% Cross Reactivity
Morphine	100
Codeine	125
Diacetylmorphine	67
Dihydrocodeine	62
Hydrocodone	59
Hydromorphone	66
Imipramine	1.6
Morphine-3-glucuronide	94
Morphine-6-glucuronide	57
6-Monoacetylmorphine	81
Meperidine	0.2
Oxymorphone	1.9
Oxycodone	3.1

High concentrations of rifampicin may cause a false positive result. A concentration of 100,000 µg/mL of floxin may cause a positive result.

Structurally unrelated compounds were tested with the CEDIA Opiate assay and gave a negative response when tested at the concentrations listed below.

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

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Opiate 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	≤ 6.0 g/dL
Glucose	≤ 3.0 g/dL		

Sensitivity

For the Qualitative application, the limit of detection (LOD) was 21.6 ng/mL.

References

- Hawks RL. Analytical methodology. In: Hawks RL, Chiang CN, eds. Urine Testing for Drugs of Abuse. NIDA Research Monograph. 1986;73:30-41.
- Balant LP, Balant-Gorgia AE. Opium and its derivatives. Clin Ther. 1992;14:846-848.
- Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals in Man. 4th ed. Foster City, Calif: Chemical Toxicology Institute; 1995.
- Glare PA, Walsh TD. Clinical pharmacokinetics of morphine. Ther Drug Monit. 1991;13:1-23.
- Cone EJ, Welch P, Paul BD, Mitchell JM. Forensic drug testing for opiates, III. Urinary excretion rates of morphine and codeine following codeine administration. J Anal Toxicol. 1991;15:161-166.
- Cone EJ, Welch P, Mitchell JM, Paul BD. Forensic drug testing for opiates, I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J Anal Toxicol. 1991;15:17.
- Mitchell JM, Paul BD, Welch P, Cone EJ. Forensic drug testing for opiates, II. Metabolism and excretion rate of morphine in humans after morphine administration. J Anal Toxicol. 1991;15:49-53.
- Hasselström J, Säwe J. Morphine Pharmacokinetics and Metabolism in Humans: Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. Clin Pharmacokinet. 1993;24:344-354.
- Henderson DR, Friedman SB, Harris JD, et al. CEDIA, a new homogeneous immunoassay system. Clin Chem. 1986;32:1637-1641.
- Ciuiti R, Quercioli M, Borsotti M. Stabilità delle principali droghe d'abuso in campioni di urine non trattate rispetto a campioni di urine stabilizzate. *Biochimica Clinica* 2014, vol. 38, n. 2.
- 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 Chimica Acta* 416: (2013) 30-35.
- C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
- 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.
- Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
- Hayes LW, Krasselt WG, Mueggler PA. Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. Clin Chem. 1987;33:806-808
- Struempfer RE. Excretion of codeine and morphine following ingestion of poppy seeds. J Anal Toxicol. 1987;11:97-99.
- 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



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.

10006508-8-EN
2019 10

thermo
scientific