

IVD For In Vitro Diagnostic Use

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

- 1 EA Reconstitution Buffer:** Contains citrate buffer, 3 μ g/mL monoclonal antibodies to opiates, 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 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

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

For preparation of the solutions for Hitachi analyzers, refer below. 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. 100098-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 analyzer/Modular analytics P system:** Use the reconstituted reagents without transfer of bottles. Discard the empty 100 mL bottles.

Cat. No. 1661248-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 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 Preparation

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 recommend that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.¹⁰

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 Calibration¹¹

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.^{12,13}
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.¹⁴ 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

Nine hundred and ninety-nine urine samples were assayed with the CEDIA Opiate assay on the Hitachi 717 analyzer using a commercially available EIA method for opiates as reference. Results were as follows:

		CEDIA	
		+	-
EIA	+	100	0
	-	0	899

Specificity

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

Compound	Concentration Tested (ng/mL)	% Cross Reactivity
Morphine	300	100
Codeine	300	125
Diacetylmorphine	300	53
Dihydrocodeine	300	50
Hydrocodone	300	48
Hydromorphone	300	57
Imipramine	20,000	1.6
Morphine-3-glucuronide	300	81
Morphine-6-glucuronide	300	47
6-Monoacetylmorphine	300	81
Meperidine	150,000	0.2
Oxymorphone	20,000	1.9
Oxycodone	10,000	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. For the Semiquantitative application, the LOD was 73.5 ng/mL.

References

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8. 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.
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11. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
12. 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
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Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342



Thermo Fisher Scientific Oy
Ratastie 2, P.O. Box 100
01621 Vantaa, Finland
Tel: +358-9-329100
Fax: +358-9-32910300



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