CEDIA™ Methadone Metabolite (EDDP) Assay

thermo scientific

IVD **For In Vitro Diagnostic Use Only**

Rx Only

REF 10016421 (3 x 17 mL Indiko Kit)
100087 (3 x 17 mL Kit)
100096 (65 mL Kit)
1868217 (495 mL Kit)

Intended Use

The CEDIA™ Methadone Metabolite Assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative assay of EDDP (2-ethylidine-1, 5-dimethyl-3, 3-diphenylpyrrolidine) in human urine. Measurements are used as an aid in the diagnosis and treatment of methadone 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

EDDP is the primary metabolite of methadone.[2] Methadone is a synthetic opiate agonist that is often used in detoxification programs as an oral substitute for heroin or other morphine-like drugs to suppress withdrawal symptoms and/or to maintain chronic relapsing heroin addicts.[3-7]

Measurement of EDDP instead of methadone for compliance can detect those individuals on the compliance program selling their methadone into the illicit drug market and spike their urine with a small quanity of methadone to cover their diversion.[8] Their urine may test positive for methadone but would not test positive for EDDP, since the drug was not ingested and therefore never metabolized.

The CEDIA Methadone Metabolite assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[9] The 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 is formed.

The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.

Reagents

1 **EA Reconstitution Buffer:** Contains piperazine-N, N-bis [2-ethanesulfonic acid], 0.66 µg/mL monoclonal antibodies to EDDP, 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-ethanesulfonic acid]; buffer salts and preservative.
2a **ED Reagent:** Contains 16.0 µg/L enzyme donor conjugated to an EDDP derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100087 and 100096 only. Refer to analyzer specific application sheet for directions on usage.) Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100096). Empty analyzer bottle for ED working solution pour-over (Cat. No. 1868217 only.)

Additional Materials Required (sold seperately):

CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Cutoffs, Secondary Cutoffs, Primary Clinical Cutoffs or Optional Cutoffs
CEDIA Multi-Drug Intermediate Calibrator
CEDIA Multi-Drug High Calibrator
CEDIA Multi-Drug Control Set, Multi-Drug Clinical Control Set, Specialty Control Set or Multi-Drug Optional Control Set

⚠ Precautions and Warnings

DANGER: Powder reagent contains ≤56% w/w bovine serum albumin (BSA), ≤2% w/w sodium azide and ≤0.5% w/w Drug-specific antibody (Mouse). 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.

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 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.100096 - 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. 1868217 - 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 Handling

Collect urine specimens in plastic or glass containers.

Specimens kept at room temperature that do not receive initial test within 7 days[10] 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 Calibration [14]

Qualitative analysis

For Qualitative evaluation of samples, use either the CEDIA Multi-Drug Calibrator, Primary Cutoffs, Secondary Cutoffs, Primary Clinical Cutoffs or Optional Cutoffs each contain 100 ng/mL EDDP. Either one can be used as a cutoff calibrator for qualitative analysis of samples. For Hitachi analyzers, place the cutoff calibrator in the appropriate standard position (S1) selected by the user. For all other analyzers, see the analyzer specific application sheet.

Semiquantitative analysis

For semiquantitative evaluation of samples, use either the CEDIA Multi-Drug Calibrator, Primary Cutoffs, Secondary Cutoffs, Primary Clinical Cutoffs or Optional Cutoffs in conjunction with the Negative, 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 selected cutoff and the other 25% below the selected cutoff. Use the CEDIA Multi-Drug Control Set, 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. Values obtained for the controls should fall within specified limits. If any trends or sudden shifts in values are detected, review all operating parameters or contact Thermo Fisher Scientific 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 cutoff calibrator 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

The Hitachi systems automatically calculate the estimated relative EDDP concentration of each sample. For all other analyzers 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 EDDP; 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).
3. High methadone sample may produce a positive EDDP result during GC/MS through thermal conversion of methaone to EDDP.[15]

Specific Performance Characteristics

Typical performance data results obtained on the Hitachi 717 analyzer are shown below.[16] 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: Within run: n=21; between day (qualitative): n=51, 1-8 runs/day for 15 days; and between day (semiquantitative): n=46, 1-6 runs/day for 18 days.

Qualitative (mA/min):

	Within-run Precision			Between day Precision		
Sample	$\bar{x}$	SD	%CV	$\bar{x}$	SD	%CV
-25% Control	244.1	2.78	1.1	244.2	5.6	2.3
Cutoff Calibrator	271.9	2.69	1.0	263.8	6.8	2.6
+ 25% Control	276.7	3.63	1.3	284.0	7.8	2.7

Semiquantitative (ng/mL):

	Within-run Precision			Between day Precision		
Sample	$\bar{x}$	SD	%CV	$\bar{x}$	SD	%CV
-25% Control	73.6	2.21	3.0	74.3	4.3	5.8
Cutoff Calibrator	90.8	3.76	4.1	100.0	4.3	4.3
+ 25% Control	122.3	4.95	4.1	130.9	6.6	5.0

Accuracy

One hundred and eighty-one samples and 19 diluted positive samples were assayed with CEDIA EDDP (100 ng/mL cutoff) qualitative assay on the Hitachi 717 using HPLC (100 ng/mL cutoff) and a commerical EIA assay (300 ng/mL cutoff) as reference. Results were as follows:

HPLC	CEDIA +	CEDIA -
+	83	4
-	2	111

EIA	CEDIA +	CEDIA -
+	80	18*
-	5†	97

* Sixteen of the eighteen discordant samples were negative by HPLC using a 100 ng/mL cutoff. The HPLC values were as follows: in six samples EDDP was not detected; the remaining results were 2, 11, 13, 42, 56, 60, 64, 86, 89 and 90 ng/mL EDDP. The HPLC values for the final two samples were 106 and 122 ng/mL EDDP.

† Three of the discordant samples resulted from the difference in cutoff concentration for the two immunoassays. The HPLC values for these samples were 104, 120 and 143 ng/mL EDDP. The HPLC values for the remaining two samples were 70 and 347 ng/mL EDDP.

Specificity

The following parent compounds and metabolites, when tested with the CEDIA EDDP assay, 100 ng/mL protocol, yielded the following cross-reactivity results:

Compound	Concentration Tested (ng/mL)	% Cross Reactivity
EDDP	100	100
EMDP	200,000	0.004
Methadone	600,000	0.016
α-levo-acetylmethadol	1,000,000	0.000
α-levo-noracetylmethadol	1,000,000	0.001
α-levo-dinoracetylmethadol	1,000,000	0.000

Structurally unrelated compounds were tested with CEDIA EDDP assay and gave a response <100 ng/mL when tested at the concentrations listed below.

Compound	Concentration Tested (ng/mL)	Compound	Concentration Tested (ng/mL)
Acetaminophen	500,000	Enalapril	500,000
Acetylsalicylic acid	500,000	Fluoxetine	500,000
Amoxicillin	500,000	Ibuprofen	500,000
Amphetamine	100,000	Levothyroxine	500,000
Benzoylecgonine	100,000	Methamphetamine	100,000
Captopril	500,000	Morphine	100,000
Chlordiazepoxide	100,000	Nifedipine	500,000
Cimetidine	500,000	Phenobarbital	100,000
Codeine	100,000	Propoxyphene	100,000
Dextromethorphan	175,000	Ranitidine	500,000
Diazepam	100,000	Salicyluric acid	500,000
Digoxin	100,000	Secobarbital	100,000
Diphenhydramine	500,000	11-nor-Δ^9-THC-COOH	9,330
Disopyramide	1,000,000	Verapamil	500,000
Doxylamine	500,000		

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA EDDP 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	≤ 1.3 g/dL
Glucose	≤ 1.0 g/dL		

Sensitivity

For the qualitative application, the limit of detection (LOD) was 6.3 ng/mL. For the semiquantitative application, the LOD was 2.0 ng/mL.

References

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12. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
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14. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
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Glossary:
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