CEDIA™ Methadone Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10016425 (3 x 17 mL Indiko Kit)
100088 (3 x 17 mL Kit)
100097 (65 mL Kit)
1730916 (495 mL Kit)

Intended Use
The CEDIA™ Methadone assay is an in-vitro diagnostic medical device intended for qualitative and semiquantitative assay of methadone 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
Methadone is a synthetic diphenylheptane-derivative opiate agonist which was first synthesized by German chemists during World War II.[2,3] It is used as an oral substitute for heroin or other morphine-like drugs to suppress withdrawal symptoms and/or to temporarily maintain chronic relapsing heroin addicts.[2-7] The pharmacological activity of methadone is very similar to that of morphine but differs in that it gives reliable effects when administered orally.[3-5,7] Its overall abuse potential is comparable to that of morphine.[4,7]

Methadone is well absorbed from the gastrointestenal tract and can be detected in plasma within 30 minutes after oral ingestion.[2,7] It binds highly to tissue protein, resulting in its cumulative effects and slow elimination.[2]

The CEDIA Methadone assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[8] 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 is 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] buffer, 3.8 mg/L mouse monoclonal antibody reactive to methadone, 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 0.0154 mg/L Enzyme Donor conjugated to methadone derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos. 100088 and 100097 only. Refer to analyzer specific application sheet for directions on usage). Empty analyzer bottle for EA/ED solution pour-over (Cat. 100097). Empty analyzer bottle for ED solution pour-over (Cat. No. 1730916 only.).

Additional Materials Required (sold separately):
CEDIA Negative Calibrator
CEDIA PPX/METD Cutoff Calibrator
CEDIA PPX/METD Intermediate Calibrator
CEDIA PPX/METD High Calibrator
CEDIA Multi-Drug Control Set

⚠ 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.

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 (20-25°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 (20-25°C). Mix again. Record the reconstitution date on the bottle label.

Cat. No. 100097 - Hitachi 717, 911, 912 or 914 anaylzer: Transfer the reconstituted reagents into the corresponding empty R1 and R2 100 mL bottles supplied with the kit. **Hitachi 917 analyzer/Modular analytics P system:** Use the reconstituted reagents without transfer of bottles. Discard the empty 100 mL bottles.
Cat. No. 1730916 - 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[9] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for two months.[10] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[10, 11]

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[13]

Qualitative assay

For **qualitative analysis** of samples, use the CEDIA PPX/METD Cutoff Calibrator to analyze results. See the analyzer specific application sheet.

Semiquantitative assay

For **semiquantitative analysis** of samples, use the CEDIA Negative Calibrator and the PPX/METD Cutoff, Intermediate, and High Calibrators, to analyze results. See the analyzer specific application sheet.

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. Recalibrate the test if reagents are changed or if control results are outside of established limits. 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 PPX/METD Cutoff Calibrator, containing 300 ng/mL methadone, 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 the analyzer specific application sheet for additional information.

Semiquantitative results

The CEDIA PPX/METD Cutoff Calibrators used in conjunction with the Negative and the PPX/METD Intermediate and High Calibrators, can be used to estimate relative concentration of methadone. 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 methadone; it does not indicate or measure intoxication.
2. Other substances and/or factors not listed may interfere with the test and cause false results (e.g., technical or procedural errors).

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.

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		
ng/mL	225	300	375
n	120	120	120
$\bar{x}$	244.4	292.8	344.0
SD	2.7	3.1	3.8
%CV	1.1	1.0	1.1

	Total Precision		
ng/mL	225	300	375
n	120	120	120
$\bar{x}$	244.4	292.8	344.0
SD	7.9	9.6	12.2
%CV	3.2	3.3	3.5

Accuracy

Eight hundred and twenty urine samples were assayed with the CEDIA Methadone assay on the Hitachi 717 analyzer using a commericial EIA method as a reference. Results were as follows:

		CEDIA +	CEDIA -
EIA	+	114	0
	-	16*	690

* Sixteen samples tested negative by EIA and positive by CEDIA, GC/MS values are as follows: 210 - 299 ng/mL in 12 of the samples, and 308 - 319 ng/mL in 4 of the samples.

Specificity

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

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
Alpha-Methadol	33,333	2.65
EDDP	500,000	0.02
EMDP	100,000	0.03
LAAM	20,000	1.48
Methadol	25,000	1.50
Methadone	300	100
Morphine-3-Glucuronide	100,000	0.01
Norpropoxyphene	500,000	0.03
Propoxyphene	500,000	0.03

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

Compound	ng/mL	Compound	ng/mL
Acetaminophen	500,000	Ibuprofen	500,000
Acetylsalicylic acid	500,000	Levothyroxine	50,000
Amoxicillin	100,000	Methamphetamine	500,000
Amphetamine	500,000	Morphine	100,000
Benzoylecgonine	500,000	Nifedipine	500,000
Captopril	500,000	Phencyclidine	500,000
Chlordiazepoxide	100,000	Phenobarbital	500,000
Cimetidine	500,000	Ranitidine	500,000
Codeine	500,000	Salicyluric acid	500,000
Diazepam	500,000	Secobarbital	500,000
Digoxin	100,000	Tolmetin	500,000
Enalapril	500,000	11-nor-Δ^9-THC-COOH	10,000
Fluoxetine	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 Methadone 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	≤ 3.0 g/dL
Glucose	≤ 3.0 g/dL		

Sensitivity

For the Qualitative application, the limit of detection (LOD) was 17.8 ng/mL.

For the Semiquantitative application, the LOD was 91.5 ng/mL.

References

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

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