CEDIA™ Barbiturate Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10017365 (3 x 17 mL Indiko Kit)
100084 (3 x 17 mL Kit)
100093 (65 mL Kit)
1661213 (495 mL Kit)

Intended Use
The CEDIA™ Barbiturate Assay is an in-vitro diagnostic medical device intended for the qualitative and semi-quantitative assay of barbiturates 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
Barbiturates belong to a broad classification of CNS-depressant drugs known as sedative/hypnotics.[2-4] When used as a substance of abuse, barbiturates are usually taken orally in pill form, but habitual users and addicts have been known to dissolve the compounds and inject them hypodermically.[2,3,5]

Depending on the degree of lipid solubility, barbiturates are commonly characterized as short, intermediate, or long acting.[2,6] Half lives range from 20 to 120 hours.[4,6] Barbiturates are variously metabolized by the liver, some being excreted in the urine mainly as active and inactive metabolites and others mainly as unchanged drug.[4,6] Depending on the specific barbiturate taken, urine may test positive for approximately 30 hours after administration or as long as several weeks.[4]

The CEDIA Barbiturate Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[7] This assay is based on the bacterial enzyme ß-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 enzyme 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], 2.2 µg/mL mouse monoclonal antibodies to barbiturates, 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 17.1 µg/L enzyme donor conjugated to a barbiturate derivative, 1.67 g/L chlorophenol red-ß-D-galactopyranoside, stabilizer and preservative.

Additional Materials:
Alternative Bar Code Labels (Cat. Nos. 100084 and 100093 only. Refer to analyzer specific application sheet for directions on usage.) Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100093). Empty analyzer bottle for ED solution pour-over (Cat. No. 1661213 only.)

Additional Materials Required (sold seperately):
CEDIA Negative Calibrator
CEDIA Multi-Drug Calibrator, Primary Cutoffs or Primary Clinical Cutoffs, 300 ng/mL
CEDIA Multi-Drug Calibrator, Secondary Cutoffs or Optional Cutoffs, 200 ng/mL
CEDIA Multi-Drug Intermediate Calibrator
CEDIA Multi-Drug High Calibrator
For 300 ng/mL Cutoff: Multi-Drug Control Set or Multi-Drug Clinical Control Set
For 200 ng/mL Cutoff: Specialty Control Set or Multi-Drug Optional 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
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.

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[8] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for up to 7 days.[9] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[10]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[11]

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[12]
Qualitative analysis
For qualitative analysis of samples, use the Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs (depending on the selected cutoff), to analyze results. See the analyzer specific application sheet.

Semi-quantitative analysis
For semi-quantitative analysis of samples, use the CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs in conjunction with the CEDIA Negative Calibrator, and the Multi-Drug Intermediate and High Calibrators to analyze results. See the analyzer specific application sheet.

Recalibrate the test if reagents are changed or if control results are outside of established limits. Each laboratory should establish its own control frequency. 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 or Multi-Drug Clinical Control Set, (300 cutoff) or Speciality Control Set or Multi-Drug Optional Control Set, (200 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. 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 CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optional Cutoffs, (depending on selected cutoffs), 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.

Semi-quantitative results
The CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Secondary Cutoffs or Optinal Cutoffs, used in conjunction with the Negative and the Multi-Drug Intermediate and High Calibrators, can be used to estimate relative concentration of barbiturates. 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 barbiturate; 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 data results obtained on the Hitachi 717 analyzer are shown below.[8] 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 following NCCLS modified replication experiment guidelines (2 runs per day, n=6/run, for 10 days).

	Within-run Precision			
ng/mL	200	225	300	375
n	120	120	120	120
$\bar{x}$	353.6	368.0	403.1	435.4
SD	5.4	4.1	3.5	4.9
CV	1.5%	1.1%	0.9%	1.1%

	Total Precision			
ng/mL	200	225	300	375
n	120	120	120	120
$\bar{x}$	353.6	368.0	403.1	435.4
SD	10.6	10.8	10.8	12.0
CV	3.0%	2.9%	2.7%	2.8%

Accuracy
Six hundred and nine samples were assayed with CEDIA Barbiturate assay on the Hitachi 717 using a commerical EIA method for barbiturates as reference. Results were as follows:

A. 200 ng/mL Cutoff

		CEDIA +	CEDIA -
EIA	+	111	0
	-	1*	497

B. 300 ng/mL Cutoff

		CEDIA +	CEDIA -
EIA	+	103	0
	-	5†	501

* The samples were tested by GC/MS and were found to contain 152 ng/mL phenobarbital.
† The samples were tested by GC/MS and were found to contain 471-1578 ng/mL phenobarbital.

Specificity
The following parent compounds and metabolites, when tested with the CEDIA Barbiturate assay, 200 ng/mL cutoff protocol, yielded the following percent cross-reactivity results:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
Secobarbital	200	100
Amobarbital	207	109
Aprobarbital	195	80
Barbital	1,000	18
Butabarbital	198	78
Butalbital	213	92
Cyclopentobarbital	190	115
Pentobarbital	270	66
Phenobarbital	195	83
Talbutal	130	160

Structurally unrelated compounds were tested with CEDIA Barbiturate assay, 200 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	Ibuprofen	500,000
Acetylsalicylic acid	500,000	Levothyroxine	50,000
Amoxicillin	100,000	Methadone	500,000
Amphetamine	500,000	Methamphetamine	500,000
Benzoylecgonine	500,000	Nifedipine	500,000
Captopril	500,000	Phencyclidine	500,000
Chlordiazepoxide	100,000	Propoxyphene	500,000
Cimetidine	500,000	Ranitidine	500,000
Diazepam	500,000	Salicyluric acid	500,000
Digoxin	100,000	11-nor-Δ^9-THC-COOH	10,000
Enalapril	500,000	Verapamil	500,000
Fluoxetine	500,000		

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA Barbiturate 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	≤ 2.0 g/dL
Glucose	≤ 1.5 g/dL		

Sensitivity
For the Qualitative application, the limit of detection (LOD), was 13.7 ng/mL and 15.2 ng/mL for the 200 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. Miller NS, Gold MS. Sedative/hypnotics. In: Giannini AJ, Slaby AE, eds. Drugs of Abuse. Oradell, NJ: Medical Economics Books, 1989.
3. Jones KL, Shainberg LW, Byer CO. Drugs and Alcohol. 3rd ed. New York, NY: Harper & Row; 1979.
4. Julien RM. A Primer of Drug Action. 6th ed. New York, NY: WH Freeman & Co; 1992.
5. Miller NS, Gold MS. Sedative-hypnotics: Pharmacology and use. J Fam Practice. 1989; 29: 665-670.
6. Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals In Man. 4th ed. Foster City, Calif.: Chemical Toxicology Institute; 1995
7. Henderson DR, Friedman SB, Harris JD, et al. CEDIA™, a new homogeneous immunoassay system. Clin Chem 1986; 32: 1637-1641.
8. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
9. Cao Z, Kaleta E, Wang P. Simultaneous Quantitation of 78 Drugs and Metabolites in Urine with a Dilute-And-Shoot LC-MS-MS Assay. *J Analytical Toxicology* 2015;39:335-346.
10. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
11. *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):119839.
12. Data on traceability are 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

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