CEDIA® Benzodiazepine Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10016409 (3 x 17 mL Indiko Kit)
100085 (3 x 17 mL Kit)
100094 (65 mL Kit)
1775561 (495 mL Kit)

Intended Use

The CEDIA® Benzodiazepine Assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative assay of benzodiazepines 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

Benzodiazepines belong to a broad classification of CNS-depressant drugs known as sedatives/hypnotics.[2] They are prescribed as anxiolytics, sleeping agents, anticonvulsants, muscle relaxers, and also widely used for preanesthetic medication and to supplement, induce, and maintain anesthesia.[2,3,4]

Although widely prescribed, benzodiazepines are also abused.[3-5] Chronic benzodiazepine use can cause physical dependence, with withdrawal symptoms of insomnia, agitation, irritability, muscle tension, and, in more severe cases, hallucinations, psychosis, and seizures.[2,3]

The CEDIA Benzodiazepine assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[6] 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.

To improve the sensitivity of the assay, an optional enzyme is added to hydrolyze glucuronide metabolites of benzodiazepines, thereby increasing the recognition of samples containing benzodiazepine metabolites.[7,8]

Reagents

1 **EA Reconstitution Buffer:** Contains Piperazine-N, N-bis [2-ethanesulfonic acid], 13.6 µg/mL sheep polyclonal antibodies to benzodiazepine, 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 9.7 µg/L Enzyme Donor conjugated to a benzodiazepine derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer, and preservative.

Additional Materials: Alternative Bar Code Labels (For Cat. Nos. 100085 and 100094. Refer to analyzer specific application sheet for directions on usage). Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100094). Empty analyzer bottle for ED solution pour-over (Cat. No. 1775561 only).

Additional materials required (sold separately):

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
Specialty Control Set, or Optional Control Set, (for 200 ng/mL cutoff)
Multi-Drug Control Set, or Clinical Control Set, (for 300 ng/mL cutoff)
β-Glucuronidase Reagent (for High Sensitivity Assay)

⚠ 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 (Sheep).
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.

Benzodiazepine High Sensitivity: To use the β-Glucuronidase reagent, add 0.09 mL of the β-Glucuronidase for Cat. No.100085 and Cat. No. 10016409, 0.325 mL for Cat. No.100094, and 2.5 mL for Cat. No. 1775561 to the reconstituted EA solution. Mix by gentle inversion. Record on the bottle label that β-Glucuronidase has been added.

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 reagent 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. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed. 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 30 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 which are 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 semiquantitative 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 CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs, (depending on the selected cutoffs) to analyze results. (For High Sensitivity application, only use Secondary Cutoff.) See the analyzer specific application sheet.

Semiquantitative analysis

For **semiquantitative analysis** of samples, use the CEDIA Multi-Drug Calibrator, Primary Cutoffs, Primary Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs, (depending on the selected cutoffs) in conjunction with the Negative Calibrator, and the Multi-Drug 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 selected cutoff; the other 25% below the selected cutoff. Use the CEDIA Multi Drug Control Set or Clinical Control Set, (300 cutoff) or Specialty Control Set,or 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. 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 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 Calibrators, Primary Cutoffs, Primary Clinical Cutoffs, Optional Cutoffs or Secondary Cutoffs, are 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 Multi-Drug Calibrator, Primary Cutoffs, 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 benzodiazepines.

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 benzodiazepines; 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 data obtained on the Hitachi 717 analyzer is shown below.[13] The results obtained in your laboratory may differ.

Precision

The following study was performed using the application with no β-Glucuronidase. The data is representative of either application.

Measured precision studies, using packaged reagents, calibrators, and control material yielded the following results in mA/min with a Hitachi 717 analyzer using NCCLS modified replication experiment (6 replicates twice daily for 10 days):

	Within-run precision				Total precision			
ng/mL	200	225	300	375	200	225	300	375
n	120	120	120	120	120	120	120	120
$\bar{x}$	324.3	340.6	366.1	402.0	324.3	340.6	366.1	402.0
SD	2.4	2.7	2.8	3.7	11.4	12.5	13.2	14.6
CV	0.8%	0.8%	0.8%	0.9%	3.5%	3.7%	3.6%	3.6%

Accuracy

Six hundred and forty-eight urine samples were assayed using the CEDIA Benzodiazepine assay on the Hitachi 717 analyzer using an EIA method as a reference (A and B). An additional two hundred clinical samples and ten spiked samples (nitrazepam spiked to within + 25% of the 200 cutoff into negative urine) were assayed with and without the addition of enzyme, β-Glucuronidase, on the Hitachi 717 analyzer (C). Results were as follows:[13]

A. 200 ng/mL Cutoff

EIA \ CEDIA	+	-
+	111	2
-	5	530

B. 300 ng/mL Cutoff

EIA \ CEDIA	+	-
+	93	2
-	14	539

C. 200 ng/mL Cutoff with Enzyme

CEDIA without enzyme \ CEDIA	+	-
+	87	0
-	6	117

Specificity

The following parent, compounds and metabolites, when tested with CEDIA Benzodiazepine Assay (without β-Glucuronidase) and High Sensitivity Assay (with β-Glucuronidase), yielded the following cross-reactivity results:

Compound	Without β-Glucuronidase 300 ng/mL cutoff		With β-Glucuronidase 200 ng/mL cutoff	
	Tested (ng/mL)	%Cross-Reactivity	Tested (ng/mL)	%Cross-Reactivity
7-Amino-Clonazepam	-	-	200	96
7-Amino-Flunitrazepam	-	-	200	99
7-Amino-Nitrazepam	-	-	250	83
α-Hydroxy-Alprazolam	163	188	115	167
α-Hydroxy-Triazolam	150	193	125	155
Alprazolam	138	205	100	220
Alprazolam glucuronide	-	-	200	100
Bromazepam	300	110	190	104
Chlordiazepoxide	2083	13	1200	16
Clobazam	400	62	300	59
Clonazepam	188	140	225	71
Clorazepate	325	84	300	75
Delorazepam	150	184	100	197
Demoxepam	1900	14	1000	19
Desalkylflurazepam	138	210	115	173
Diazepam	110	247	125	154
Estazolam	125	220	95	239
Flunitrazepam	188	135	175	109
Flurazepam	150	189	100	195
Halazepam	200	145	200	101
Lorazepam	208	122	175	115
Lorazepam glucuronide	10000	1	400	45
Lormetazepam	163	165	150	137
Medazepam	200	135	150	118
Nitrazepam	300	100	200	97
Nordiazepam	150	211	120	173
Oxaprozin	10000	2	10000	2
Oxazepam	275	107	165	125
Oxazepam glucuronide	10000	1	800	25
Prazepam	150	184	160	116
Temazepam	175	144	180	93
Temazepam glucuronide	10000	1	750	25
Triazolam	138	191	90	217

Structurally unrelated compounds were tested with the CEDIA Benzodiazepine assay, 300 ng/mL cutoff protocol, and gave a negative response when tested at the concentrations listed below. Similar performance is seen using the High Sensitivity 200 ng/mL cutoff protocol.

Compound	ng/mL	Compound	ng/mL
11-nor-Δ^9-THC-COOH	10,000	Levothyroxine	50,000
Acetaminophen	500,000	Methadone	100,000
Acetylsalicylic acid	500,000	Methamphetamine	500,000
Amoxicillin	100,000	Morphine	100,000
Amphetamine	500,000	Nifedipine	500,000
Benzoylecgonine	500,000	Phencyclidine	250,000
Captopril	500,000	Phenobarbital	500,000
Cimetidine	500,000	Propoxyphene	500,000
Codeine	500,000	Ranitidine	500,000
Digoxin	100,000	Salicyluric acid	500,000
EDDP	500,000	Secobarbita	500,000
EMDP	100,000	Sertraline	250,000
Enalapril	500,000	Tolmetin	500,000
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 Benzodiazepine assay:

Substance	Concentration	Substance	Concentration
Acetone	≤ 1.0 g/dL	Hemoglobin	≤ 0.3 g/dL
Ascorbic acid	≤ 0.15 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

Standard application

For the Qualitative application, the limit of detection (LOD) was 10.8 ng/mL and 12.8 ng/mL for the 200 ng/mL and 300 ng/mL cutoff protocols, respectively. For the Semiquantitative application, the LOD was 6.4 ng/mL and 8.3 ng/mL for the 200 ng/mL and 300 ng/mL cutoff protocols, respectively.

Benzodiazepine High Sensitivity application

For the qualitative application, the LOD was 12.3 ng/mL. For the semiquantitative application, LOD was 7.3 ng/mL.

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. Katzung BG, ed. Basic and clinical pharmacology. 5th ed. Norwalk, Conn: Appleton & Lange, 1992.
3. Julien RM.. A primer of drug action. 6th ed. New York, NY: W.H. Freeman & Co; 1992.
4. Goodman and Gilman's The Pharmacological basis of therapeutics. 8th ed. New York, NY: Pergamon Press, 1990.
5. Adams EH. Prevalence of prescription drug abuse: Data from the National Institute on Drug Abuse. NY State J Med 199; 91 (suppl 11): 32s-36s.
6. Henderson DR, Friedman SB, Harris JD et al. CEDIA™, a new homogeneous immunoassay system. Clin Chem. 1986; 32: 1637-1641.
7. Beck O, Lafolle P, Hjemdahl P et al. Detection of Benzodiazepine Intake in Therapeutic Doses by Immunoanalysis of Urine: Two Techniques Evaluated and Modified for Improved Performance. Clin Chem. 1992; 38: 271-275.
8. Simonsson P, Liden A, Lindberg S. Effect of a-Glucuronidase on Urinary Benzodiazepine Concentrations Determined by Flourescence Polarization Immunossay. Clin Chem. 1995; 41: 920-923.
9. Dixon RB, Mbeunkui F, Wiegel JW. Stability study of opioids and benzodiazepines in urine samples by liquid chromatography tandem mass spectrometry. *Journal of Analytical Science and Technology* (2015) 6:17.
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):11983.
12. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
13. 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

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

For insert updates go to:
www.thermoscientific.com/diagnostics

Other countries:

Please contact your local Thermo Fisher Scientific representative.

10006458-7-EN
2017 12

Thermo SCIENTIFIC