CEDIA™ Benzodiazepine Assay

thermo scientific

For Criminal Justice and Forensic Use Only

REF 1775561-CJF (495 mL Kit)
100085-CJF (3 x 17 mL Kit)
100094-CJF (1 x 65 mL Kit)

Intended Use

The CEDIA™ Benzodiazepine Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semi-quantitative determination of benzodiazepines in human urine at a cutoff concentration of either 200 ng/mL or 300 ng/mL.

The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/tandem mass spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

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) or Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the preferred confirmatory method.[1] Professional judgement should be applied to any drug of abuse test result particularly when preliminary positive results are used.

The CEDIA Benzodiazepine Assay is for **Criminal Justice & Forensic use only** – Intended for the justice system. This product is **not intended** for clinical diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae or for patient management.

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 detect benzodiazepine glucuronide, add β-glucuronidase enzyme to the reconstituted EA solution. This enzyme will hydrolyze the glucuronidated metabolites of benzodiazepines in the samples, thereby enabling the detection of benzodiazepine glucuronides.[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, 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 Beckman Coulter AU680 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.

Samples were prepared by spiking nitrazepam into drug free urine at cutoff (100%), 25%, 50%, 75% and 100% above and below the cutoff and tested in duplicate (n=2) twice per day for 20 days (total n=80 for each level), in both qualitative and semi-quantitative modes. The results of the Precision study is shown below.

200 ng/mL cutoff

Spiked Concentration (ng/mL)	% of cutoff (200 ng/mL)	Total Precision (n=80)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-quantitative Immunoassay Results (Negative/Positive)
0	-100	80	80/0	80/0
50	-75	80	80/0	80/0
100	-50	80	80/0	80/0
150	-25	80	80/0	80/0
200	100	80	73/7	62/18
250	+25	80	0/80	0/80
300	+50	80	0/80	0/80
350	+75	80	0/80	0/80
400	+100	80	0/80	0/80

300 ng/mL cutoff

Spiked Concentration (ng/mL)	% of cutoff (300 ng/mL)	Total Precision (n=80)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-quantitative Immunoassay Results (Negative/Positive)
0	-100	80	80/0	80/0
75	-75	80	80/0	80/0
150	-50	80	80/0	80/0
225	-25	80	80/0	80/0
300	100	80	78/2	80/0
375	+25	80	0/80	0/80
450	+50	80	0/80	0/80
525	+75	80	0/80	0/80
600	+100	80	0/80	0/80

Accuracy

One hundred and ten samples were tested on the Beckman Coulter AU680 clinical chemistry analyzer and confirmed by LC-MS/MS for 200 ng/mL and high sensitivity 200 ng/mL cutoff. One hundred and thirteen samples were tested and confirmed by LC-MS/MS for 300 ng/mL cutoff. The results are presented below.

Qualitative and Semi-Quantitative Accuracy Study with LC-MS/MS as Reference Method - 200 ng/mL Cutoff

Candidate Device Results	< 50% of Cutoff concentration by LC-MS/MS (< 100 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (100 – 199.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (200 – 300 ng/mL)	High Positives (Greater than 50% above cutoff concentration) (> 300 ng/mL)
Positive	0	*2	5	45
Negative	55	3	0	0

* Discordant Result Table for Discrepant Samples

Sample ID	EIA		LC-MS/MS
	Qualitative Mode	Semi-quantitative Mode	Total Benzodiazepine Parent Only (ng/mL)
CA160606-045	Positive	Positive	148.86
CA170605-001	Positive	Positive	182.42

These 2 samples are discordant due to the presence of Benzodiazepine metabolites.
Sample CA160606-045 contains 3154.59 ng/mL of 7-aminoclonazepam
Sample CA170605-001 contains 560.37 ng/mL of 7-aminoclonazepam, and 1.43 ng/mL of α-hydroxyalprazolam

Qualitative and Semi-Quantitative Accuracy Study with LC-MS/MS as Reference Method - 300 ng/mL Cutoff

Candidate Device Results	< 50% of Cutoff concentration by LC-MS/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (150 – 299.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (300 – 450 ng/mL)	High Positives (Greater than 50% above cutoff concentration) (> 450 ng/mL)
Positive	**1	**2	5	45
Negative	56	4	0	0

** Discordant Result Table for Discrepant Samples

Sample ID	EIA		LC-MS/MS
	Qualitative Mode	Semi-quantitative Mode	Total Benzodiazepine Parent Only (ng/mL)
CA160606-045	Positive	Positive	117.61
CA170605-001	Positive	Positive	175.19
CA160926-057	Positive	Positive	213.9

These 3 samples are discordant due to the presence of Benzodiazepine metabolites.
Sample CA160606-045 contains 3154.59 ng/mL of 7-aminoclonazepam
Sample CA170605-001 contains 560.37 ng/mL of 7-aminoclonazepam, and 1.43 ng/mL of α-hydroxyalprazolam
Sample CA160926-057 contains 410.69 ng/mL of 7-aminoclonazepam, and 13.46 ng/mL of α-hydroxyalprazolam

Qualitative and Semi-Quantitative Accuracy Study with LC-MS/MS as Reference Method - High Sensitivity 200 ng/mL Cutoff

Candidate Device Results	< 50% of Cutoff concentration by LC-MS/MS (< 100 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (100 – 199.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (200 – 300 ng/mL)	High Positives (Greater than 50% above cutoff concentration) (> 300 ng/mL)
Positive	0	†2	6	45
Negative	54	3	0	0

† Discordant Result Table for Discrepant Samples

Sample ID	EIA		LC-MS/MS
	Qualitative Mode	Semi-quantitative Mode	Total Benzodiazepine Parent Only (ng/mL)
CA160606-045	Positive	Positive	135.68
CA170605-001	Positive	Positive	175

These 2 samples are discordant due to the presence of Benzodiazepine metabolites.
Sample CA170605-001 contains 560.37 ng/mL of 7-aminoclonazepam, and 1.43 ng/mL of α-hydroxyalprazolam
Sample CA160606-045 contains 3154.59 ng/mL of 7-aminoclonazepam

Analytical Recovery and Dilution Linearity

Five replicates of each level indicated below were tested in semi-quantitative mode, and the average was used to determine percent recovery compared to the expected target value. The assay demonstrated linearity from 0 - 800 ng/mL.

Dilution Linearity for 200 ng/mL and 300 ng/mL Cutoff

Level	Target Concentration (ng/mL)	Observed Concentration (ng/mL)	Recovery (%)
1	0	-1	N/A
2	100	115.6	115.60
3	200	198	99.00
4	300	304.4	101.47
5	400	448.6	112.15
6	500	580.8	116.16
7	600	657	109.50
8	700	732.6	104.66
9	800	950.4	118.80

Specificity

The following benzodiazepines and metabolites, when tested with CEDIA Benzodiazepine Assay 200 ng/mL and 300 ng/mL cutoff (without β-Glucuronidase) and High Sensitivity 200 ng/mL Assay (with β-Glucuronidase), yielded the following cross-reactivity results:

Cross Reactivity of Benzodiazepines and Metabolites - 200 ng/mL Cutoff

Benzodiazepines and metabolites	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
α-Hydroxyalprazolam	115	Positive	174
α-Hydroxytriazolam	100	Positive	200
Alprazolam	80	Positive	250
7-Aminoclonazepam	515	Positive	39
7-Aminoflunitrazepam	150	Positive	133
7-Aminonitrazepam	375	Positive	53
Bromazepam	250	Positive	80
Chlordiazepoxide	1100	Positive	18
Clobazam	345	Positive	58
Clonazepam	300	Positive	67
Clorazepate	110	Positive	182
Delorazepam	100	Positive	200
Demoxepam	1500	Positive	13
Desalkylflurazepam (Norfludiazepam)	115	Positive	174
Diazepam	105	Positive	190
Estazolam	95	Positive	211
Flunitrazepam	131.25	Positive	152
Flurazepam	85	Positive	235
Lorazepam	175	Positive	114
Lorazepam glucuronide	10000	Negative	< 2
Lormetazepam	150	Positive	133
Medazepam	140	Positive	143
Nitrazepam	200	Positive	100
Nordiazepam (Desmethyldiazepam)	95	Positive	211
Oxazepam	165	Positive	121
Oxazepam glucuronide	10000	Negative	< 2
Prazepam	96	Positive	208
Temazepam	135	Positive	148
Temazepam glucuronide	10000	Negative	< 2
Triazolam	100	Positive	200

Cross Reactivity of Benzodiazepines and Metabolites - 300 ng/mL Cutoff

Benzodiazepines and metabolites	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
α-Hydroxyalprazolam	115	Positive	261
α-Hydroxytriazolam	135	Positive	222
Alprazolam	100	Positive	300
7-Aminoclonazepam	900	Positive	33
7-Aminoflunitrazepam	225	Positive	133
7-Aminonitrazepam	800	Positive	38
Bromazepam	450	Positive	67
Chlordiazepoxide	2600	Positive	12
Clobazam	650	Positive	46
Clonazepam	600	Positive	50
Clorazepate	135	Positive	222
Delorazepam	150	Positive	200
Demoxepam	2755	Positive	11

Table continued

Benzodiazepines and metabolites	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
Desalkylflurazepam (Norfludiazepam)	138	Positive	217
Diazepam	115	Positive	261
Estazolam	115	Positive	261
Flunitrazepam	188	Positive	160
Flurazepam	120	Positive	250
Lorazepam	325	Positive	92
Lorazepam glucuronide	10000	Negative	< 3
Lormetazepam	200	Positive	150
Medazepam	170	Positive	176
Nitrazepam	300	Positive	100
Nordiazepam (Desmethyldiazepam)	125	Positive	240
Oxazepam	275	Positive	109
Oxazepam glucuronide	10000	Negative	< 3
Prazepam	125	Positive	240
Temazepam	175	Positive	171
Temazepam glucuronide	10000	Negative	< 3
Triazolam	130	Positive	231

Cross Reactivity of Benzodiazepines and Metabolites - High Sensitivity 200 ng/mL Cutoff

Benzodiazepines and metabolites	Tested Concentration (ng/mL)	Positive/ Negative	Cross-reactivity (%)
α-Hydroxyalprazolam	115	Positive	174
α-Hydroxytriazolam	100	Positive	200
Alprazolam	80	Positive	250
7-Aminoclonazepam	515	Positive	39
7-Aminoflunitrazepam	160	Positive	125
7-Aminonitrazepam	450	Positive	44
Bromazepam	250	Positive	80
Chlordiazepoxide	1100	Positive	18
Clobazam	400	Positive	50
Clonazepam	300	Positive	67
Clorazepate	110	Positive	182
Delorazepam	100	Positive	200
Demoxepam	1500	Positive	13
Desalkylflurazepam (Norfludiazepam)	115	Positive	174
Diazepam	105	Positive	190
Estazolam	95	Positive	211
Flunitrazepam	175	Positive	114
Flurazepam	85	Positive	235
Lorazepam	200	Positive	100
Lorazepam glucuronide	320	Negative	63
Lormetazepam	150	Positive	133
Medazepam	140	Positive	143
Nitrazepam	210	Positive	95
Nordiazepam (Desmethyldiazepam)	95	Positive	211
Oxazepam	175	Positive	114
Oxazepam glucuronide	320	Negative	63
Prazepam	96	Positive	208
Temazepam	135	Positive	148
Temazepam glucuronide	245	Positive	82
Triazolam	100	Positive	200

Structurally unrelated compounds and/or concurrently used drugs were evaluated by adding each substance to nitrazepam spiked at low (150 ng/mL for 200 ng/mL cutoff, and 225 ng/mL for 300 ng/mL cutoff) and high (250 ng/mL for 200 ng/mL cutoff, and 375 ng/mL for 300 ng/mL cutoff) controls at the concentration indicated. As shown in the tables below, the Controls were detected accurately, Low Control as Negative and High Control as Positive for both 200 ng/mL and 300 ng/mL cutoffs, indicating that all the compounds evaluated exhibited minimal cross-reactivity at the concentrations tested.

Structurally Unrelated Compounds Spiked into Low and High Controls - 200 ng/mL and 300 ng/mL Cutoff

Structurally Unrelated Compounds	Tested Concentration (ng/mL)	200 ng/mL cutoff		300 ng/mL cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
6-Acetyl Morphine	100000	Negative	Positive	Negative	Positive
10,11 Dihydrocarbamazepine	100000	Negative	Positive	Negative	Positive
11-nor-Δ^9-THC-COOH	100000	Negative	Positive	Negative	Positive
Acetaminophen	100000	Negative	Positive	Negative	Positive
Acetylsalicylic Acid	100000	Negative	Positive	Negative	Positive
Amitriptyline	75000	Negative	Positive	Negative	Positive
Amoxicillin	100000	Negative	Positive	Negative	Positive
Amphetamine	100000	Negative	Positive	Negative	Positive
Benzoylecgonine	100000	Negative	Positive	Negative	Positive
Brompheniramine	100000	Negative	Positive	Negative	Positive
Buprenorphine	100000	Negative	Positive	Negative	Positive
Caffeine	100000	Negative	Positive	Negative	Positive
Captopril	100000	Negative	Positive	Negative	Positive
Cimetidine	100000	Negative	Positive	Negative	Positive
Codeine	100000	Negative	Positive	Negative	Positive
Desipramine	100000	Negative	Positive	Negative	Positive
Dextromethorphan	100000	Negative	Positive	Negative	Positive
Digoxin	100000	Negative	Positive	Negative	Positive
Diphenhydramine	50000	Negative	Positive	Negative	Positive
EDDP	100000	Negative	Positive	Negative	Positive
EMDP	15000	Negative	Positive	Negative	Positive
Fentanyl	100000	Negative	Positive	Negative	Positive
Fluoxetine	75000	Negative	Positive	Negative	Positive
Fluphenazine	75000	Negative	Positive	Negative	Positive
Haloperidol	100000	Negative	Positive	Negative	Positive
Heroin	100000	Negative	Positive	Negative	Positive
Hydrocodone	100000	Negative	Positive	Negative	Positive
Hydromorphone	100000	Negative	Positive	Negative	Positive
Ibuprofen	100000	Negative	Positive	Negative	Positive
Levorphanol	100000	Negative	Positive	Negative	Positive
Meperidine	100000	Negative	Positive	Negative	Positive
Methadone	75000	Negative	Positive	Negative	Positive
Methamphetamine	100000	Negative	Positive	Negative	Positive
Morphine	100000	Negative	Positive	Negative	Positive
Morhpine-3β-D-glucuronide	100000	Negative	Positive	Negative	Positive
Morhpine-6β-D-glucuronide	100000	Negative	Positive	Negative	Positive
Nalbuphine	100000	Negative	Positive	Negative	Positive
Nalorphine	100000	Negative	Positive	Negative	Positive
Naloxone	100000	Negative	Positive	Negative	Positive
Naltrexone	100000	Negative	Positive	Negative	Positive
Naproxen	100000	Negative	Positive	Negative	Positive
Nifedipine	100000	Negative	Positive	Negative	Positive
Oxycodone	100000	Negative	Positive	Negative	Positive

Table Continued

Structurally Unrelated Compounds	Tested Concentration (ng/mL)	200 ng/mL cutoff		300 ng/mL cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
Oxymorphone	100000	Negative	Positive	Negative	Positive
Perphenazine	50000	Negative	Positive	Negative	Positive
Phencyclidine	100000	Negative	Positive	Negative	Positive
Phenobarbital	100000	Negative	Positive	Negative	Positive
Procyclidine	100000	Negative	Positive	Negative	Positive
Propoxyphene	100000	Negative	Positive	Negative	Positive
Ranitidine	100000	Negative	Positive	Negative	Positive
Secobarbital	100000	Negative	Positive	Negative	Positive
Sertraline	15000	Negative	Positive	Negative	Positive
Sulpiride	100000	Negative	Positive	Negative	Positive
Tapentadol	100000	Negative	Positive	Negative	Positive
Thioridazine	100000	Negative	Positive	Negative	Positive
Tramadol	100000	Negative	Positive	Negative	Positive
Triprolidine	50000	Negative	Positive	Negative	Positive
Verapamil	100000	Negative	Positive	Negative	Positive
Zolpidem	50000	Negative	Positive	Negative	Positive
Enalapril	100000	Negative	Positive	Negative	Positive
Salicyluric Acid	100000	Negative	Positive	Negative	Positive
Tolmetin	100000	Negative	Positive	Negative	Positive

Structurally Unrelated Compounds Spiked into Low and High Controls - High Sensitivity 200 ng/mL Cutoff

Structurally Unrelated Compounds	Tested Concentration (ng/mL)	High Sensitivity 200 ng/mL Cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)
6-Acetyl Morphine	100000	Negative	Positive
10,11 Dihydrocarbamazepine	100000	Negative	Positive
11-nor-Δ^9-THC-COOH	100000	Negative	Positive
Acetaminophen	100000	Negative	Positive
Acetylsalicylic Acid	100000	Negative	Positive
Amitriptyline	75000	Negative	Positive
Amoxicillin	100000	Negative	Positive
Amphetamine	100000	Negative	Positive
Benzoylecgonine	100000	Negative	Positive
Brompheniramine	100000	Negative	Positive
Buprenorphine	100000	Negative	Positive
Caffeine	100000	Negative	Positive
Captopril	100000	Negative	Positive
Cimetidine	100000	Negative	Positive
Codeine	100000	Negative	Positive
Desipramine	100000	Negative	Positive
Dextromethorphan	100000	Negative	Positive
Digoxin	100000	Negative	Positive
Diphenhydramine	50000	Negative	Positive
EDDP	100000	Negative	Positive
EMDP	15000	Negative	Positive
Fentanyl	100000	Negative	Positive
Fluoxetine	75000	Negative	Positive
Fluphenazine	75000	Negative	Positive
Haloperidol	100000	Negative	Positive
Heroin	100000	Negative	Positive

Table Continued

Structurally Unrelated Compounds	Tested Concentration (ng/mL)	High Sensitivity 200 ng/mL Cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)
Hydrocodone	100000	Negative	Positive
Hydromorphone	100000	Negative	Positive
Ibuprofen	100000	Negative	Positive
Levorphanol	100000	Negative	Positive
Meperidine	100000	Negative	Positive
Methadone	75000	Negative	Positive
Methamphetamine	100000	Negative	Positive
Morphine	100000	Negative	Positive
Morhpine-3β-D-glucuronide	100000	Negative	Positive
Morhpine-6β-D-glucuronide	100000	Negative	Positive
Nalbuphine	100000	Negative	Positive
Nalorphine	100000	Negative	Positive
Naloxone	100000	Negative	Positive
Naltrexone	100000	Negative	Positive
Naproxen	100000	Negative	Positive
Nifedipine	100000	Negative	Positive
Oxycodone	100000	Negative	Positive
Oxymorphone	100000	Negative	Positive
Perphenazine	50000	Negative	Positive
Phencyclidine	100000	Negative	Positive
Phenobarbital	100000	Negative	Positive
Procyclidine	100000	Negative	Positive
Propoxyphene	100000	Negative	Positive
Ranitidine	100000	Negative	Positive
Secobarbital	100000	Negative	Positive
Sertraline	15000	Negative	Positive
Sulpiride	100000	Negative	Positive
Tapentadol	100000	Negative	Positive
Thioridazine	100000	Negative	Positive
Tramadol	100000	Negative	Positive
Triprolidine	50000	Negative	Positive
Verapamil	100000	Negative	Positive
Zolpidem	50000	Negative	Positive
Enalapril	100000	Negative	Positive
Salicyluric Acid	100000	Negative	Positive
Tolmetin	100000	Negative	Positive

Interference

The potential interference of endogenous, exogenous, physiological substances, and pH on the recovery of nitrazepam using CEDIA Benzodiazepine Assay was assessed. Potentially interfering substances were spiked into the low (150 ng/mL for 200 ng/mL cutoff, and 225 ng/mL for 300 ng/mL cutoff) and high (250 ng/mL for 200 ng/mL cutoff, and 375 ng/mL for 300 ng/mL cutoff) controls at the concentration indicated. As shown in the tables below, the Controls were detected accurately, Low Control as Negative and High Control as Positive for both 200 ng/mL and 300 ng/mL cutoffs, indicating that all these compounds did not show interference in the assay.

Compounds	Tested Conc. (mg/dL)	200 ng/mL cutoff		300 ng/mL cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
Acetaminophen	10	Negative	Positive	Negative	Positive
Acetone	500	Negative	Positive	Negative	Positive
Acetyl Salicylic Acid	10	Negative	Positive	Negative	Positive
Ascorbic Acid	150	Negative	Positive	Negative	Positive

Table continued

Compounds	Tested Conc. (mg/dL)	200 ng/mL cutoff		300 ng/mL cutoff	
		Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
Caffeine	5	Negative	Positive	Negative	Positive
Creatinine	400	Negative	Positive	Negative	Positive
Ethanol	1000	Negative	Positive	Negative	Positive
Galactose	5	Negative	Positive	Negative	Positive
Glucose	1000	Negative	Positive	Negative	Positive
Hemoglobin	150	Negative	Positive	Negative	Positive
Human Serum Albumin	200	Negative	Positive	Negative	Positive
Ibuprofen	10	Negative	Positive	Negative	Positive
Oxalic acid	50	Negative	Positive	Negative	Positive
Riboflavin	3	Negative	Positive	Negative	Positive
Sodium Chloride	1000	Negative	Positive	Negative	Positive
Urea	1000	Negative	Positive	Negative	Positive

pH	200 ng/mL cutoff		300 ng/mL cutoff	
	Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
5	Negative	Positive	Negative	Positive
6	Negative	Positive	Negative	Positive
7	Negative	Positive	Negative	Positive
8	Negative	Positive	Negative	Positive
9	Negative	Positive	Negative	Positive
10	Negative	Positive	Negative	Positive

Specific Gravity

Drug free urine samples with specific gravity ranging in value from 1.006 to 1.032 were split and spiked with nitrazepam to a final concentration of either 150 ng/mL, 250 ng/mL (for 200 ng/mL cutoff), 225 ng/mL, 375 ng/mL (for 300 ng/mL cutoff). These samples were then evaluated in qualitative and semi-quantitative modes. The Controls were detected accurately, indicating no interference was observed.

Specific gravity	200 ng/mL cutoff		300 ng/mL cutoff	
	Low Control (150 ng/mL)	High Control (250 ng/mL)	Low Control (225 ng/mL)	High Control (375 ng/mL)
1.006	Negative	Positive	Negative	Positive
1.007	Negative	Positive	Negative	Positive
1.008	Negative	Positive	Negative	Positive
1.011	Negative	Positive	Negative	Positive
1.013	Negative	Positive	Negative	Positive
1.015	Negative	Positive	Negative	Positive
1.015	Negative	Positive	Negative	Positive
1.020	Negative	Positive	Negative	Positive
1.026	Negative	Positive	Negative	Positive
1.028	Negative	Positive	Negative	Positive
1.032	Negative	Positive	Negative	Positive

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13. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

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