CEDIA® Buprenorphine II Assay

thermo scientific

IVD **For *In Vitro* Diagnostic Use Only**

Rx Only

REF 10020849 (3 x 17 mL Kit)
10020850 (65 mL Kit)

Intended Use

The CEDIA® Buprenorphine II Assay is a homogeneous enzyme immunoassay for the qualitative and/or semi-quantitative determination for the presence of buprenorphine and its metabolites in human urine at a cut-off concentration of 10 ng/mL. The assay is intended to be used in laboratories and provides a simple and rapid analytical screening procedure to detect buprenorphine and its metabolites in human urine. The assay is designed for use with a number of clinical chemistry analyzers.

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]

Clinical and professional judgment should be applied to any drugs of abuse test result, particularly when preliminary results are used. For *In Vitro Diagnostic* Use Only.

Summary and Explanation of the Test

Buprenorphine is a semi-synthetic opioid analgesic derived from thebaine, a minor component of opium. Buprenorphine is structurally similar to morphine. It is a partial agonist receptor modulator.[2] Buprenorphine has a longer duration of action than morphine and can be administered sublingually as an analgesic. Subutex®, a higher dose buprenorphine formulation, is widely used in Europe and elsewhere as a substitution treatment for opiate addiction.[3-5] The FDA has approved the use of Subutex and Suboxone®, containing buprenorphine as the active drug, for the treatment of opiate dependence in the US. Subutex and Suboxone are the first narcotic drugs available under the US Drug Abuse Treatment Act (DATA) of 2003 for the treatment of opiate dependence that can be prescribed in the US in a physician's work place.[6] It has also been shown that buprenorphine has abuse potential and may cause dependency. In addition, a number of deaths have been recorded as a result of overdose with intravenously injected buprenorphine in conjunction with other psychotropic drugs such as benzodiazepines.[7] Buprenorphine is metabolized primarily by N-dealkylation to form norbuprenorphine and by conjugation to form buprenorphine-glucuronide and norbuprenorphine-glucuronide.[8]

The CEDIA Buprenorphine II Assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system.[9] The assay is based on the bacterial β-galactosidase enzyme *(Escherichia coli)*, which has been genetically engineered into two inactive fragments. These fragments spontaneously re-associate to form fully active enzymes that, in the assay format, cleave a substrate, generating a color change that can be measured spectrophotometrically at 570 nm.

In this assay, the analyte in the sample competes with the analyte conjugated to the inactive fragment (enzyme donor) of the β-galactosidase enzyme for the antibody binding site. If the analyte is present in the sample, it binds to the antibody, leaving the inactive enzyme fragment free to form active enzyme. If the analyte is not present in the sample, antibody binds to the analyte conjugated on the inactive fragment, inhibiting the re-association of inactive β-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change is directly proportional to the amount of analyte present in the sample.

Reagents

1 EA Reconstitution Buffer
Contains buffer salts, mouse monoclonal anti-buprenorphine derivative antibody 0.8 - 1.0 mg/L, stabilizer, and preservative.

1a EA Reagent
Contains 0.171 g/L Enzyme Acceptor, buffer salts and preservative.

2 ED Reconstitution Buffer
Contains buffer salts, stabilizers, and preservatives

2a ED Reagent
Contains 0.175 mg/L Enzyme Donor conjugated to buprenorphine derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizers, detergent and preservative.

Additional Materials Required (sold separately):

REF	Kit Description
10021390	CEDIA Negative Calibrator II (1 x 7.5 mL)
10020799	CEDIA Buprenorphine II Calibrator 10 ng/mL (1 x 5 mL)
10020800	CEDIA Buprenorphine II Calibrator 20 ng/mL (1 x 5 mL)
10020801	CEDIA Buprenorphine II Calibrator 50 ng/mL (1 x 5 mL)
10020802	CEDIA Buprenorphine II Calibrator 100 ng/mL (1 x 5 mL)
10020804	CEDIA Buprenorphine II Low (7.5 ng/mL) and High (12.5 ng/mL) Controls (2 x 5 mL each)

⚠ Warnings and Precautions

DANGER: Powder reagents contain ≤55% w/w Bovine Serum Albumin (BSA) fragments and ≤1% w/w Sodium Azide. Liquid reagents contain ≤0.5% Bovine Serum, ≤0.2% Sodium Azide, and ≤0.1% Drug-Specific Antibody (Mouse).

The reagents are harmful if swallowed.

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 mist or vapor. 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 a doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

In the case of accidental spill, clean and dispose of material according to your laboratory's Standard Operating Procedure (SOP), local, and state regulations.

In the case of damaged packaging on arrival, contact your technical support representative (refer to last page of this Package Insert).

Reagent Preparation and Storage

For preparation of the solutions, refer to the section below. Remove the kit from refrigerated storage (2-8°C) 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 (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

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 (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

⚠ **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 caps to the proper reagent bottles. The R2 solution (Enzyme Donor) should be yellow-orange in color. A red or red-purple color indicates that the reagent has been contaminated and must be discarded. Discard Reagents 1 or 2 if turbidity or precipitates are observed.

⚠ **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.

Store reagents at 2-8°C. **DO NOT FREEZE.**

For shelf life 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 8 days[10] of arrival at the laboratory should be placed into a secure refrigeration unit at 2-8°C for up to 30 days.[11,12] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[13] Studies have shown buprenorphine analytes in urine are stable at -20°C up to 85 days.[13]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[14]

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

The CEDIA Buprenorphine II Assay is intended for use on automated clinical analyzers capable of maintaining a constant temperature, pipetting, mixing reagents, measuring enzymatic rates at 570 nm and timing the reaction accurately can be used to perform this immunoassay.
Refer to specific application instructions for each analyzer for chemistry parameters before performing the assay.

Qualitative analysis

For qualitative analysis, use the CEDIA Buprenorphine II cutoff calibrator (10 ng/mL).

Semi-quantitative analysis

For semi-quantitative analysis, use all five calibrators.

Quality Control and Calibration

Good laboratory practice requires the use of control specimens to ensure proper assay performance. Ensure that control results are within the established ranges, as determined by laboratory procedures and guidelines. If results fall outside of the established ranges, assay results are invalid. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements. Each laboratory should establish its own quality control testing frequency.

Results and Expected values

Qualitative

The 10 ng/mL calibrator is used as a cutoff reference for distinguishing 'positive' from 'negative' samples. A sample that exhibits a change in absorbance values (Δ A) equal to or greater than that obtained with the cutoff calibrator is considered as positive. A sample that exhibits a change in absorbance value (Δ A) lower than that obtained with the cutoff calibrator is considered as negative.

Semi-quantitative

An estimate of drug concentrations in the samples can be obtained by running a standard curve with all calibrators and estimating sample concentrations off the standard curve. Sample results above the high calibrator should be diluted with negative urine calibrator and retested.

Limitations

1. A positive result from this assay indicates only the presence of Buprenorphine or its metabolites and does not necessarily correlate with the extent of physiological and psychological effects. This is a screening test. All positive results must be confirmed via GC/MS or LC-MS/MS.
2. It is possible that substances other than those investigated in the specificity study may interfere with the test and cause false results.
3. Care should be taken when reporting concentration results since there are many factors e.g. fluid intake and other biologic factors that may influence a urine test result.
4. Performance characteristics for the CEDIA Buprenorphine II assay performance have not been established with body fluids other than human urine.

Specific Performance Characteristics

Typical performance results obtained on a Beckman Coulter AU680 analyzer are shown below. The results obtained in your laboratory may differ from these data.

Precision

Samples were prepared by spiking Burprenorphine into drug free urine at the cutoff, 25%, 50%, 75% & 100% above and below the cutoff and tested in both qualitative and semi-quantitative modes using a Clinical Laboratory and Standards Institute (CLSI) protocol. Results presented below were generated by testing all samples in replicates of 2, twice per day for 20 days, total n=80. Representative data is presented below.

Qualitative Study Analysis

Buprenorphine Spike Concentration (ng/mL)	% of Cutoff (10 ng/mL)	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			Number of Determinations	Immunoassay Results (Negative/Positive)
0	-100%	0.00	80	80/0
2.5	-75%	2.99	80	80/0
5	-50%	5.31	80	80/0
7.5	-25%	7.63	80	80/0
10	100%	10.99	80	27/53
12.5	+25%	12.97	80	0/80
15	+50%	15.05	80	0/80
17.5	+75%	18.92	80	0/80
20	+100%	20.38	80	0/80

Semi-Quantitative Study Analysis

Buprenorphine Spike Concentration (ng/mL)	% of Cutoff (10 ng/mL)	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			Number of Determinations	Immunoassay Results (Negative/Positive)
0	-100%	0.00	80	80/0
2.5	-75%	2.99	80	80/0
5	-50%	5.31	80	80/0
7.5	-25%	7.63	80	80/0
10	100%	10.99	80	35/45
12.5	+25%	12.97	80	0/80
15	+50%	15.05	80	0/80
17.5	+75%	18.92	80	0/80
20	+100%	20.38	80	0/80

Accuracy

One hundred and fifty three urine patient samples were analyzed by the CEDIA Buprenorphine II Assay in both qualitative and semi-quantitative modes and the results were compared to LC-MS/MS.

Qualitative Accuracy study with LC-MS/MS for Buprenorphine only as reference method

Candidate Device Results	Negative	< 50% of Cutoff concentration by LC-MS/MS (< 5 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (5 – 9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (10 – 15.0 ng/mL)	High Positives (Greater than 50% above cutoff concentration) (> 15.0 ng/mL)
Positive	31*	11*	4*	5	45
Negative	49	2	6	0	0

Semi-Quantitative Accuracy study with LC-MS/MS for Buprenorphine only as reference method

Candidate Device Results	Negative	< 50% of Cutoff concentration by LC-MS/MS (< 5 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (5 – 9.9 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (10 – 15.0 ng/mL)	High Positives (Greater than 50% above cutoff concentration) (> 15.0 ng/mL)
Positive	32 *ǂ	11*	4*	5	45
Negative	48	2	6	0	0

Table for Discordant Samples

Accuracy samples were categorized based upon the LC-MS/MS concentration for Buprenorphine only. The table below identifies those samples with Buprenorphine concentration below the cutoff, in which the observed CEDIA Buprenorphine II assay result was positive due to detection of Buprenorphine metabolites.

	EIA		LC-MS/MS Concentration (ng/mL)				
Sample ID	Qualitative Mode	SQ (ng/mL)	Bup***	NorBup#	Bup-Glu†	NorBup-Glu‡	Total by LC-MS/MS
51	Pos	10.08	<LLOQ**	2.27	1.96	6.18	10.41
52	Pos	10.02	<LLOQ	0.69	3.15	6.84	10.68
53╫	Neg	10.42	<LLOQ	1.08	7.89	1.82	10.79
54	Pos	11.59	<LLOQ	1.09	5.67	5.54	12.30
55	Pos	10.40	<LLOQ	3.27	2.54	7.92	13.73
56	Pos	16.36	<LLOQ	4.02	7.46	3.73	15.21
57	Pos	17.31	<LLOQ	3.28	10.67	3.09	17.04
58	Pos	19.82	<LLOQ	5.03	10.91	2.05	17.99
59	Pos	18.73	<LLOQ	3.10	9.09	6.59	18.78
60	Pos	22.63	<LLOQ	4.18	8.30	7.34	19.82
61	Pos	18.95	<LLOQ	1.96	9.90	9.90	21.76
62	Pos	26.11	<LLOQ	4.36	10.87	6.92	22.15
63	Pos	24.99	<LLOQ	5.26	8.41	9.01	22.68
64	Pos	24.91	<LLOQ	3.86	23.19	<LLOQ	27.05
65	Pos	20.87	<LLOQ	1.44	14.06	14.06	29.56
66	Pos	23.21	<LLOQ	2.23	25.24	2.50	29.97
67	Pos	30.27	<LLOQ	4.42	8.82	16.84	30.08
68	Pos	31.35	<LLOQ	16.52	9.41	5.47	31.40
69	Pos	35.38	<LLOQ	7.13	5.30	22.38	34.81
70	Pos	40.38	<LLOQ	12.21	18.65	9.11	39.97
71	Pos	38.44	<LLOQ	2.93	12.40	28.84	44.17
72	Pos	48.60	<LLOQ	23.41	15.34	5.44	44.19
73	Pos	62.31	<LLOQ	5.47	36.52	25.00	66.99
74	Pos	81.31	<LLOQ	33.59	23.42	12.72	69.73
75	Pos	88.67	<LLOQ	26.22	32.43	23.10	81.75
76	Pos	79.26	<LLOQ	6.34	80.00	2.77	89.11
77	Pos	>100.01	<LLOQ	8.63	56.89	46.95	112.47
78	Pos	>100.01	<LLOQ	101.98	10.40	9.90	122.28
79	Pos	>100.01	<LLOQ	7.91	26.43	144.00	178.34
80	Pos	>100.01	<LLOQ	49.66	97.61	121.12	268.39
81	Pos	>100.01	<LLOQ	<LLOQ	145.72	394.81	540.53
82	Pos	>100.01	<LLOQ	129.95	105.07	664.47	899.49
83	Pos	>100.01	0.81	32.14	39.52	59.14	131.61
84	Pos	63.54	0.86	7.41	29.46	31.38	69.11
85	Pos	20.48	0.90	5.42	11.54	<LLOQ	17.86
86	Pos	>100.01	0.91	54.00	18.10	10.52	83.53
87	Pos	46.32	2.00	12.03	13.58	16.24	43.85
88	Pos	>100.01	2.00	6.83	193.42	131.65	333.90
89	Pos	>100.01	2.02	75.75	174.74	442.98	695.49
90	Pos	66.32	2.48	6.53	57.67	1.52	68.20
91	Pos	>100.01	3.63	80.26	733.7	624.02	1441.61
92	Pos	>100.01	4.38	69.28	146.16	349.33	569.15
93	Pos	>100.01	4.45	59.03	55.01	17.31	135.80
100	Pos	>100.01	8.64	36.91	>ULOQ**	224.42	>1000
101	Pos	>100.01	8.94	51.32	497.32	55.06	612.64
102	Pos	>100.01	5.22	35.13	85.99	22.24	148.58
103	Pos	77.36	6.60	147.58	195.67	40.28	390.13

** <LLOQ: Lower Limit of Quantitation (0.65 ng/mL), >ULOQ: Upper Limit of Quantitation (1000 ng/mL);
*** Bup: Buprenorphine;
# NorBup: Norbuprenorphine;
† Bup-Glu: Buprenorphine-β-D-glucuronide;
‡ NorBup-Glu: Norbuprenorphine-β-D-glucuronide;
╫ Additional discordant sample for Semi-Quantitative mode

Analytical Recovery and Dilution Linearity

To demonstrate the dilution linearity for purposes of sample dilution and quality control of the entire assay range, drug free urine was spiked to the high calibrator level of Buprenorphine (100 ng/mL) and diluted with drug free urine to generate 10 intermediate levels. Each sample was run in replicates of 5 in semi-quantitative mode and the average was used to determine percent recovery compared to the expected target value. Representative data is presented below.

Buprenorphine		Recovery (%)
Target Concentration (ng/mL)	Observed Concentration (ng/mL)	
5	5.99	119.8
10	10.97	109.7
20	19.66	98.3
30	33.03	110.1
40	43.83	109.6
50	52.98	106.0
60	67.28	112.1
70	77.54	110.8
80	85.14	106.4
90	95.38	106.0
100	104.70	104.7

Specificity

The cross-reactivity of Buprenorphine and its metabolites was evaluated by adding known amounts of each analyte to drug free urine. As indicated by the results in the table below, Buprenorphine, Norbuprenorphine and Norbuprenorphine-glucuronide exhibited ≥ 100% cross-reactivity. Buprenorphine-glucuronide showed less cross-reactivity.

Buprenorphine and its metabolites	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
Buprenorphine	10	Pos	100
Norbuprenorphine	8	Pos	125
Buprenorphine-β-D-glucuronide	13	Pos	76.9
Norbuprenorphine-β-D-glucuronide	10	Pos	100

Cross-reactivity of structurally related or unrelated opiate compounds

Structurally related compounds and other opiates	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
6-Acetyl morphine	100,000	Neg	< 0.01
Diacetylmorphine (Heroin)	100,000	Neg	< 0.01
Codeine	100,000	Neg	< 0.01
Dextromethorphan	100,000	Neg	< 0.01
Dihydrocodeine	100,000	Neg	< 0.01
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	100,000	Neg	< 0.01
EMDP (2-Ethyl-5-methyl-3,3-diphenylpyrroline)	100,000	Neg	< 0.01
Fentanyl	100,000	Neg	< 0.01
Hydrocodone	100,000	Neg	< 0.01
Hydromorphone	100,000	Neg	< 0.01
Hydromorphone-β-D-glucuronide	10,000	Neg	< 0.1
LAAM (Levo-alpha-acetylmethadol)	100,000	Neg	< 0.01
Levorphanol	100,000	Neg	< 0.01
Methadone	100,000	Neg	< 0.01
Meperidine	100,000	Neg	< 0.01
Morphine	100,000	Neg	< 0.01
Morphine-3β-D-glucuronide	100,000	Neg	< 0.01
Morphine-6β-D-glucuronide	100,000	Neg	< 0.01
Nalorphine	100,000	Neg	< 0.01

Table continued

Structurally related compounds and other opiates	Tested Concentration (ng/mL)	Pos/Neg	Cross-reactivity (%)
Naloxone	100,000	Neg	< 0.01
Naltrexone	100,000	Neg	< 0.01
Norcodeine	100,000	Neg	< 0.01
Norhydrocodone	100,000	Neg	< 0.01
Norpropoxyphene	100,000	Neg	< 0.01
Noroxycodone	100,000	Neg	< 0.01
Noroxymorphone	100,000	Neg	< 0.01
Oxymorphone-β-D-glucuronide	10,000	Neg	< 0.1
Oxycodone	100,000	Neg	< 0.01
Oxymorphone	100,000	Neg	< 0.01
Tapentadol	100,000	Neg	< 0.01
Tramadol	100,000	Neg	< 0.01

The potential cross-reactivity posed by drugs commonly co-administered with Buprenorphine was evaluated by adding each substance to Buprenorphine spiked at low (7.5 ng/mL) and high (12.5 ng/mL) controls at the concentrations indicated. A drug was considered to cross-react if the observed Buprenorphine concentrations result exceeded 10 ng/mL. As shown in the table below, all the pharmacologic compounds evaluated exhibited minimal cross reactivity at the concentrations tested.

Structurally unrelated compounds spiked at the concentration listed below into Low and High controls

Cross-reactants	Spiked Concentration (ng/mL)	Spiked Buprenorphine Level	
		Low Control	High Control
Acetaminophen	500,000	Neg	Pos
Acetylsalicylic acid	500,000	Neg	Pos
Amitryptyline	50,000	Neg	Pos
Amoxicillin	100,000	Neg	Pos
Amphetamine	1,000,000	Neg	Pos
Amisulpride	100,000	Neg	Pos
Benzoylecgonine	1,000,000	Neg	Pos
Caffeine	100,000	Neg	Pos
Carbamazepine	100,000	Neg	Pos
Chlorpromazine	100,000	Neg	Pos
Clomipramine	25,000	Neg	Pos
Chloroquine	100,000	Neg	Pos
Cimetidine	500,000	Neg	Pos
Desipramine	10,000	Neg	Pos
Doxepine	25,000	Neg	Pos
Diphenylhydramine	100,000	Neg	Pos
Ephedrine	100,000	Neg	Pos
Fluoxethine	100,000	Neg	Pos
Fluphenazine	100,000	Neg	Pos
Hydroxychloroquine	100,000	Neg	Pos
Ibuprofen	100,000	Neg	Pos
Imipramine	25,000	Neg	Pos
Maprotiline	100,000	Neg	Pos
Mitragynine	100,000	Neg	Pos
7-hydroxymitragynine	10,000	Neg	Pos
Nalbuphine	100,000	Neg	Pos
Nortryptiline	50,000	Neg	Pos
Oxazepam	100,000	Neg	Pos
Phencyclidine	100,000	Neg	Pos
Phenobarbital	100,000	Neg	Pos

Table continued

Cross-reactants	Spiked Concentration (ng/mL)	Spiked Buprenorphine Level	
		Low Control	High Control
Ranitidine	500,000	Neg	Pos
Secobarbital	100,000	Neg	Pos
Sulpiride	100,000	Neg	Pos
Thioridazine	100,000	Neg	Pos
Trimipramine	25,000	Neg	Pos

Interference

The potential interference of pH and endogenous physiologic substances on recovery of Buprenorphine using CEDIA Buprenorphine II Assay was assessed by spiking known compounds of potentially interfering substances into the low (7.5 ng/mL) and high (12.5 ng/mL) controls for 10 ng/mL cutoff. In the presence of the compounds listed below, the controls were detected accurately, indicating that these compounds did not show interference in the assay.

Compound	Tested Concentration (mg/dL)	Spiked Buprenorphine Level	
		Low Control	High Control
Acetaminophen	10	Neg	Pos
Acetone	500	Neg	Pos
Acetyl Salicylic Acid	10	Neg	Pos
Ascorbic Acid	150	Neg	Pos
Caffeine	10	Neg	Pos
Creatinine	400	Neg	Pos
Ethanol	10	Neg	Pos
Galactose	5	Neg	Pos
Glucose	1000	Neg	Pos
Hemoglobin	150	Neg	Pos
Human Serum Albumin	200	Neg	Pos
Ibuprofen	10	Neg	Pos
Oxalic acid	50	Neg	Pos
Riboflavin	3	Neg	Pos
Sodium Chloride	1000	Neg	Pos
Urea	1000	Neg	Pos
pH	3	Neg	Pos
pH	4	Neg	Pos
pH	5	Neg	Pos
pH	6	Neg	Pos
pH	7	Neg	Pos
pH	8	Neg	Pos
pH	9	Neg	Pos
pH	10	Neg	Pos
pH	11	Neg	Pos

Specific Gravity

Drug free urine samples with specific gravity ranging in value from 1.002 to 1.030 were split and spiked to a final concentration of either 7.5 ng/mL or 12.5 ng/mL (the low and high control concentrations, respectively). These samples were then evaluated in qualitative and semi-quantitative modes. No interference was observed.

Specific Gravity	Spiked Buprenorphine Level	
	Low Control	High Control
1.002	Neg	Pos
1.004	Neg	Pos
1.008	Neg	Pos
1.013	Neg	Pos
1.016	Neg	Pos
1.018	Neg	Pos
1.022	Neg	Pos
1.023	Neg	Pos
1.025	Neg	Pos
1.030	Neg	Pos

References

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