

LZI Fentanyl III Enzyme Immunoassay

For Beckman Coulter, Inc.

REF D29739 (100/37.5 mL R₁/R₂ Kit)

C82916 (1000/375 mL R₁/R₂ Kit)



IVD For In Vitro Diagnostic Use Only



Lin-Zhi International, Inc.

Intended Use

The LZI Fentanyl III Enzyme Immunoassay for Beckman Coulter, Inc. is intended for the qualitative determination of fentanyl in human urine at the cutoff value of 1 ng/mL when calibrated against fentanyl. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The assay provides only a preliminary analytical result. A more specific alternative chemical method (e.g., gas or liquid chromatography and mass spectrometry) must be used in order to obtain a confirmed analytical result. (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Fentanyl is an important opioid analgesic used widely in surgical operations and is a controlled substance (3). Fentanyl is most commonly encountered in the form of patches applied to the skin, as "lollipops" which can be dissolved in the mouth through the mucous membrane, or can be administered intravenously. It is 50-100 times stronger than morphine (4, 5) and cases of fentanyl abuse via intravenous injection, inhalation, oral, or nasal applications have been previously reported (6). Fentanyl is used in the treatment of acute and chronic pain, usually in patients who no longer respond to high doses of less potent opioids such as morphine or oxycodone. Due to its potency and wide availability as a prescribed drug, fentanyl has been abused and misused by health professionals, pain management patients, and recreational abusers (7).

Due to its short elimination half-life and approximately 90% metabolism, fentanyl is difficult to detect in urine (8). Fentanyl undergoes extensive hepatic biotransformation to metabolites coming from hydrolysis, N-dealkylation, or hydroxylation reactions (9). In an intravenous dose of fentanyl, up to 85% is excreted in urine over a three- to four- day period with 0.4-6% eliminated as unchanged fentanyl and 26-55% eliminated as the norfentanyl metabolite (10).

Fentanyl analogs also have high potency analgesic activities. Numerous reports have been published with modified fentanyl-related compounds abused as designer drugs (11-13).

Other recently available fentanyl analogs associated with abuse and severe intoxication include butyryl fentanyl and 4-fluorobutyryl fentanyl (14-18).

Assay Principle

The LZI Fentanyl III assay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (19). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, fentanyl-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody would bind to free drug; the unbound fentanyl-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R₁): Contains a mouse monoclonal anti-fentanyl antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09%) as a preservative.

Enzyme-drug Conjugate Reagent (R₂): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with fentanyl in buffer with sodium azide (0.09%) as a preservative.

Calibrators and Controls*

Materials required (but not provided)

*Calibrators and Controls are sold separately and contain negative human urine with sodium azide as a preservative.

Qualitative Calibration	REF
LZI Fentanyl Qualitative Calibrator FEN Cutoff Calibrator (1 ng/mL)	D26784

Controls	REF
LZI Fentanyl Level 1 Control FEN Level 1 Control (0.5 ng/mL)	D26785
LZI Fentanyl Level 2 Control FEN Level 2 Control (1.5 ng/mL)	D26786

Others

WEDGE	REF
OSR Bottle kit, 20 x 60 mL	63093
OSR Bottle kit, 20 x 30 mL	63094

Precautions and Warning

- This test is for in vitro diagnostic use only. Harmful if swallowed.
- Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes, always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (20).
- Do not use the reagents beyond their expiration dates.
- Rx ONLY** For USA: Cautions: Federal law restricts this device to sale by or on the order of a physician.

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8°C when not in use. See expiration date on individual bottle labels.

Specimen Collection and Handling

Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8°C for up to four weeks (21, 22) or at room temperature for up to four weeks (21, 22). For longer storage, keep sample frozen at -20°C and then thaw before use. Studies have shown fentanyl samples in urine are stable at -20°C for up to six months (23). Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Samples should be equilibrated to room temperature (18-25°C) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to a laboratory for testing.

Handle all urine specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Beckman Coulter AU5800.

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Additional wash steps may be required. Please refer to the specific parameters used for each analyzer before performing the assay.

For qualitative analysis use the 1 ng/mL as the cutoff calibrator. The cutoff is normalized to 100. Positive samples are ≥ 100 and are flagged with a (P). Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 0.5 ng/mL and 1.5 ng/mL.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact your local Beckman Coulter Representative for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took a specific drug and a negative test result does not necessarily mean a person did not take a specific drug. There are a number of factors that influence the reliability of drug tests.

Normal populations have a value below the cutoff. Affected populations have a value above the cutoff.

Qualitative: The cutoff calibrator, which contains 1 ng/mL of fentanyl, is used as a reference for distinguishing a preliminary positive from negative samples.

Samples producing a positive or "0" absorbance value are considered preliminary positive. Samples producing a negative absorbance value are considered negative. Results of this assay distinguish preliminary positive (≥ 1 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Limitations

- Boric Acid at 1% w/v may cause false negative results. Boric Acid is not recommended as a preservative for urine.*
- Dextromethorphan may cause false positive results at concentrations greater than 15,000 ng/mL.*
- A preliminary positive result from the assay indicates the possible presence of fentanyl or cross-reactive substances. This test is not intended for quantifying the individual analytes in samples.*
- A preliminary positive result does not necessarily indicate drug abuse.*
- A negative result does not necessarily mean a person did not take illegal drugs.*
- Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
- Preliminary positive results should be confirmed by other affirmative, analytical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
- The test is designed for use with human urine only.*
- The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU5800 automated chemistry analyzer.

Precision:

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by AOD, mAU) are as follows:

1 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
Fentanyl Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0%	22	22 Neg	88	88 Neg
0.25 ng/mL	25%	22	22 Neg	88	88 Neg
0.50 ng/mL	50%	22	22 Neg	88	88 Neg
0.75 ng/mL	75%	22	22 Neg	88	88 Neg
1.0 ng/mL	100%	22	22 Pos	88	4 Neg/ 84 Pos
1.25 ng/mL	125%	22	22 Pos	88	88 Pos
1.50 ng/mL	150%	22	22 Pos	88	88 Pos
1.75 ng/mL	175%	22	22 Pos	88	88 Pos
2.0 ng/mL	200%	22	22 Pos	88	88 Pos

Accuracy: One-hundred and fifty (150) unaltered clinical urine specimens were tested with the LZ1 Fentanyl III Enzyme Immunoassay and confirmed by LC/MS. Specimens having a fentanyl concentration greater than or equal to 1 ng/mL by LC/MS are defined as positive, and specimens with fentanyl concentrations below 1 ng/mL by LC/MS are defined as negative in the table below. Near cutoff samples are defined as $\pm 50\%$ of the cutoff value. The correlation results are summarized as follows:

Qualitative Accuracy Study:

FEN Results 1 ng/mL Cutoff		LC/MS				
		Neg	< 50% below 50% of the cutoff concentration	Near Cutoff Negative between 50% below the cutoff and the cutoff concentration	Near Cutoff Positive between the cutoff and 50% above the cutoff concentration	High Positive greater than 50% above the cutoff concentration
EIA	Positive at or above the cutoff	0	20*	12**	22	61
	Negative below the cutoff	35	0	0	0	0

The following table summarizes the results for the qualitative discrepant samples:

Sample #	FEN LC/MS (ng/mL)	NFEN LC/MS (ng/mL)	LC/MS Pos/Neg Result	AU5800 EIA Qualitative Result (mAU)	AU5800 EIA Qualitative Cutoff Rate (mAU)	EIA Pos/Neg Result
36*	0.0	1.5	-	83.7	19.3	+
37*	0.0	4.1	-	172.3	19.3	+
38*	0.1	1.1	-	57.3	19.3	+
39*	0.1	6.7	-	203.9	19.3	+
40*	0.1	4.1	-	234.2	19.3	+
41*	0.1	4.4	-	192.7	19.3	+
42*	0.1	6.1	-	134.5	19.3	+
43*	0.1	5.3	-	150.6	19.3	+
44*	0.1	4.4	-	104.9	19.3	+
45*	0.2	4.8	-	206.2	19.3	+
46*	0.2	1.9	-	214.7	19.3	+
47*	0.2	2.0	-	151.3	19.3	+
48*	0.2	5.1	-	101.1	19.3	+
49*	0.2	5.0	-	206.2	19.3	+
50*	0.2	39.9	-	213.6	22.1	+
51*	0.2	10.0	-	155.3	19.3	+
52*	0.3	2.4	-	171.4	19.3	+
53*	0.3	9.7	-	253.1	19.3	+
54*	0.3	4.2	-	166.2	19.3	+
55*	0.3	5.4	-	162.5	19.3	+
56**	0.6	2.2	-	185.6	19.3	+
57**	0.6	362.5	-	225.1	19.3	+
58**	0.7	15.9	-	228.2	22.1	+
59**	0.7	101.7	-	246.8	22.1	+
60**	0.7	167.1	-	260.2	19.3	+
61**	0.8	30.2	-	314.7	19.3	+
62**	0.8	2.8	-	45.0	22.1	+
63**	0.8	0.5	-	108.2	19.3	+
64**	0.8	2.8	-	134.8	19.3	+
65**	0.9	15.8	-	224.3	19.3	+
66**	0.9	106.7	-	318.7	22.1	+
67**	0.9	12.0	-	258.4	19.3	+

* Discrepant below 50% of the cutoff concentration (0.0 ng/mL - 0.5 ng/mL)

* Discrepant between -50% of the cutoff concentration and the cutoff concentration (0.5 ng/mL - 1.0 ng/mL)

These discrepant samples contained levels of norfentanyl that contributed to the false positive result.

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix to various concentrations and evaluated against the cutoff calibrator.

The following table lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative).

Compounds tested at high concentration with results below the cutoff value were listed as Not Detected (ND).

Fentanyl and Metabolites:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity	Result
Fentanyl	1.0	100.00%	Positive
Norfentanyl	2.5	40.00%	Positive

Structurally Related Compounds:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity
4-Fluoro-isobutyl fentanyl	25	4.00%
9-Hydroxy risperidone	100,000	ND
Acetyl fentanyl	4	25.00%
Acetyl norfentanyl	100	1.00%
Acryl Fentanyl	1	100.00%
Alfentanil	100,000	ND
Benzyl fentanyl	2	50.00%
Butyl fentanyl	1	100.00%
Butyl norfentanyl	40	2.50%
Carfentanil oxalate	100,000	ND
(±) cis-3-methyl fentanyl	4.5	22.22%
Cyclopropyl norfentanyl	15	6.67%
Despropionyl fentanyl (4-ANPP)	100,000	ND
Furanyl fentanyl	2.5	40.00%
Furanyl norfentanyl	150	0.67%
β-Hydroxyfentanyl	1.1	90.91%
(±)-β-Hydroxythiofentanyl	1	100.00%
Isobutyl fentanyl	12.5	8.00%
Isobutyl norfentanyl	390	0.26%
Methoxyacetyl fentanyl	1	100.00%
MT-45	100,000	ND
N-benzyl furanyl norfentanyl	0.75	133.33%
N-benzyl para-fluoro norfentanyl	1	100.00%
Norcarfentanil oxalate	100,000	ND
Ocfentanil	1	100.00%
Para-fluorobutyl fentanyl	1.2	83.33%
Para-fluoro fentanyl	1	100.00%
Para-methoxy-butyl fentanyl	1	100.00%
Remifentanil	100,000	ND
Risperidone	100,000	ND
Sufentanil	1,000	0.10%
Thienyl fentanyl	0.75	133.33%
Thiofentanyl	0.50	200.00%
Trans-3-methyl fentanyl	2.0	50.00%
Trazodone	100,000	ND
U-47700	100,000	ND
Valeryl Fentanyl	50	2.00%
Ortho-methyl fentanyl	3.5	28.57%

Structurally Unrelated Compounds:

If discrepant results were observed, the lowest tested concentration at which discrepancies occurred is presented in the following table. Dextromethorphan was found to cause interference with the assay.

Compound	Spiked (ng/mL)	Spiked Fentanyl Concentration		
		0 ng/mL	0.5 ng/mL Control	1.5 ng/mL Control
(1S,2S)-(+)-Pseudoephedrine	100,000	Neg	Neg	Pos
6-Acetylmorphine	100,000	Neg	Neg	Pos
7-Hydroxymitragynine	100,000	Neg	Neg	Pos
Acetaminophen	100,000	Neg	Neg	Pos
Acetylsalicylic acid	100,000	Neg	Neg	Pos
AH 7921	100,000	Neg	Neg	Pos
Alprazolam	100,000	Neg	Neg	Pos
Amitriptyline	100,000	Neg	Neg	Pos
Amlodipine besylate	100,000	Neg	Neg	Pos
Amobarbital	100,000	Neg	Neg	Pos
Amoxicillin	100,000	Neg	Neg	Pos
d-Amphetamine	100,000	Neg	Neg	Pos
Aripiprazole	100,000	Neg	Neg	Pos
Atorvastatin	100,000	Neg	Neg	Pos
Benzoylcegonine	100,000	Neg	Neg	Pos
Bisoprolol	100,000	Neg	Neg	Pos
Bromazepam	100,000	Neg	Neg	Pos
Buprenorphine glucuronide	100,000	Neg	Neg	Pos
Bupropion	100,000	Neg	Neg	Pos
Butalbital	100,000	Neg	Neg	Pos
Caffeine	100,000	Neg	Neg	Pos
Cannabidiol	100,000	Neg	Neg	Pos
Carbamazepine	100,000	Neg	Neg	Pos
Carisoprodol	100,000	Neg	Neg	Pos
Cetirizine	100,000	Neg	Neg	Pos
Chlordiazepoxide	100,000	Neg	Neg	Pos
Chlorpheniramine	100,000	Neg	Neg	Pos
Chlorpromazine	100,000	Neg	Neg	Pos
Clobazam	100,000	Neg	Neg	Pos
Clomipramine	100,000	Neg	Neg	Pos
Clonazepam	100,000	Neg	Neg	Pos
Cocaine	100,000	Neg	Neg	Pos
Codeine	100,000	Neg	Neg	Pos
Cotinine	100,000	Neg	Neg	Pos
Cyclobenzaprine	100,000	Neg	Neg	Pos
Desipramine	100,000	Neg	Neg	Pos
Dextromethorphan	20,000	Neg	Pos	Pos

Structurally Unrelated Compounds, continued:

Compound	Spiked (ng/mL)	Spiked Fentanyl Concentration		
		0 ng/mL (ng/mL)	0.5 ng/mL Control (ng/mL)	1.5 ng/mL Control (ng/mL)
Diazepam	100,000	Neg	Neg	Pos
Diphenhydramine	100,000	Neg	Neg	Pos
Doxepin	100,000	Neg	Neg	Pos
Doxylamine	100,000	Neg	Neg	Pos
Duloxetine	100,000	Neg	Neg	Pos
EDDP	100,000	Neg	Neg	Pos
Ecgonine	100,000	Neg	Neg	Pos
Ecgonine methyl ester	100,000	Neg	Neg	Pos
EMDP	100,000	Neg	Neg	Pos
Ephedrine	100,000	Neg	Neg	Pos
Fexofenadine	100,000	Neg	Neg	Pos
Flunitrazepam	5,000	Neg	Neg	Pos
Fluoxetine	100,000	Neg	Neg	Pos
Fluphenazine	100,000	Neg	Neg	Pos
Flurazepam	100,000	Neg	Neg	Pos
Furosemide	100,000	Neg	Neg	Pos
Gabapentin	100,000	Neg	Neg	Pos
Haloperidol	100,000	Neg	Neg	Pos
Heroin	100,000	Neg	Neg	Pos
Hexobarbital	100,000	Neg	Neg	Pos
Hydrochlorothiazide	100,000	Neg	Neg	Pos
Hydrocodone	100,000	Neg	Neg	Pos
Hydromorphone	100,000	Neg	Neg	Pos
Ibuprofen	100,000	Neg	Neg	Pos
Imipramine	100,000	Neg	Neg	Pos
Ketamine	100,000	Neg	Neg	Pos
Labetalol	100,000	Neg	Neg	Pos
Lamotrigine	100,000	Neg	Neg	Pos
Levorphanol	100,000	Neg	Neg	Pos
Lidocaine	100,000	Neg	Neg	Pos
Lisinopril	100,000	Neg	Neg	Pos
Loratadine	100,000	Neg	Neg	Pos
Lormetazepam	100,000	Neg	Neg	Pos
Losartan	100,000	Neg	Neg	Pos
LSD	100,000	Neg	Neg	Pos
Maprotiline	100,000	Neg	Neg	Pos
mCPP	100,000	Neg	Neg	Pos
MDA	100,000	Neg	Neg	Pos
MDEA	100,000	Neg	Neg	Pos
MDMA	100,000	Neg	Neg	Pos
Meperidine	100,000	Neg	Neg	Pos
Mephobarbital	100,000	Neg	Neg	Pos
Meprobamate	100,000	Neg	Neg	Pos
Metformin	100,000	Neg	Neg	Pos
Methadone	100,000	Neg	Neg	Pos
d-Methamphetamine	100,000	Neg	Neg	Pos
Methapyrilene	100,000	Neg	Neg	Pos
Methaqualone	100,000	Neg	Neg	Pos
Methylphenidate	100,000	Neg	Neg	Pos
Metoprolol	100,000	Neg	Neg	Pos
Metronidazole	100,000	Neg	Neg	Pos
Minocycline	100,000	Neg	Neg	Pos
Mirtazapine	100,000	Neg	Neg	Pos
Morphine	100,000	Neg	Neg	Pos
Morphine-3-glucuronide	100,000	Neg	Neg	Pos
Nalmefene	100,000	Neg	Neg	Pos
Naloxone	100,000	Neg	Neg	Pos
Naltrexone	100,000	Neg	Neg	Pos
Naproxen	100,000	Neg	Neg	Pos
Nicotine	100,000	Neg	Neg	Pos
Nitrazepam	100,000	Neg	Neg	Pos
Norbuprenorphine	100,000	Neg	Neg	Pos
Norcodeine	100,000	Neg	Neg	Pos
Norketamine	100,000	Neg	Neg	Pos
Normeperidine	100,000	Neg	Neg	Pos
Normorphine	100,000	Neg	Neg	Pos
Norquetiapine	100,000	Neg	Neg	Pos
Nortriptyline	100,000	Neg	Neg	Pos
Olanzapine	100,000	Neg	Neg	Pos
Omeprazole	100,000	Neg	Neg	Pos
Oxazepam	100,000	Neg	Neg	Pos
Oxycodone	100,000	Neg	Neg	Pos
Oxymorphone	100,000	Neg	Neg	Pos
Paroxetine	100,000	Neg	Neg	Pos
Pentazocine	100,000	Neg	Neg	Pos
Pentobarbital	100,000	Neg	Neg	Pos
Perphenazine	100,000	Neg	Neg	Pos
Phencyclidine	100,000	Neg	Neg	Pos
Phenobarbital	100,000	Neg	Neg	Pos
Phentermine	100,000	Neg	Neg	Pos
Propoxyphene	100,000	Neg	Neg	Pos

Structurally Unrelated Compounds, continued:

Compound	Spiked [] (ng/mL)	Spiked Fentanyl Concentration		
		0 ng/mL (ng/mL)	0.5 ng/mL Control (ng/mL)	1.5 ng/mL Control (ng/mL)
Quetiapine	100,000	Neg	Neg	Pos
Quinidine	100,000	Neg	Neg	Pos
Ranitidine	100,000	Neg	Neg	Pos
Ritalinic acid	100,000	Neg	Neg	Pos
Phenytoin	100,000	Neg	Neg	Pos
Salbutamol	100,000	Neg	Neg	Pos
Salicylic acid	100,000	Neg	Neg	Pos
Secobarbital	100,000	Neg	Neg	Pos
Sertraline	100,000	Neg	Neg	Pos
Sildenafil	100,000	Neg	Neg	Pos
Tapentadol	100,000	Neg	Neg	Pos
Temazepam	100,000	Neg	Neg	Pos
THC	100,000	Neg	Neg	Pos
Thebaine	100,000	Neg	Neg	Pos
Theophylline	100,000	Neg	Neg	Pos
Thioridazine	100,000	Neg	Neg	Pos
L-Thyroxine	100,000	Neg	Neg	Pos
Tilidine	100,000	Neg	Neg	Pos
Tramadol	100,000	Neg	Neg	Pos
Trazadone	100,000	Neg	Neg	Pos
Triazolam	100,000	Neg	Neg	Pos
Trimipramine	100,000	Neg	Neg	Pos
Valproic acid	100,000	Neg	Neg	Pos
Verapamil	100,000	Neg	Neg	Pos
Venlafaxine	100,000	Neg	Neg	Pos
Ziprasidone	100,000	Neg	Neg	Pos
Zolpidem	100,000	Neg	Neg	Pos
Zopiclone	100,000	Neg	Neg	Pos

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results

The following structurally unrelated compound which showed interference at $\pm 50\%$ of the cutoff concentration was then tested at the highest concentration that did not cause a discrepant result. The result is summarized in the following table:

Compound	Spiked [] (ng/mL)	Spiked Fentanyl Concentration		
		0 ng/mL (ng/mL)	0.5 ng/mL Control (ng/mL)	1.5 ng/mL Control (ng/mL)
Dextromethorphan	15,000	Neg	Neg	Pos

Endogenous and Preservatives Compound Interference Study:

Various potentially interfering endogenous and preservative substances were tested for interference with the assay. Test compounds were split into three portions each and either left un-spiked or spiked to a fentanyl concentration of either 0.5 or 1.5 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. Boric Acid (1% w/v) was found to cause interference with the assay. Results are summarized in the following table:

Endogenous or Preservative Substance	Spiked [] (mg/dL)	Spiked Fentanyl Concentration		
		0 ng/mL	0.5 ng/mL Control	1.5 ng/mL Control
Acetone	1,000	Neg	Neg	Pos
Ascorbic acid	500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Biotin	2	Neg	Neg	Pos
Boric acid	1,000	Neg	Neg	Neg
Calcium chloride	300	Neg	Neg	Pos
Citric Acid	200	Neg	Neg	Pos
Creatinine	500	Neg	Neg	Pos
Ethanol	1,000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ -Globulin	500	Neg	Neg	Pos
Glucose	3,000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
Human urine (pooled)	N/A	Neg	Neg	Pos
Human serum albumin	500	Neg	Neg	Pos
β -Hydroxybutyric acid	100	Neg	Neg	Pos
Oxalic acid	100	Neg	Neg	Pos
Potassium chloride	1,000	Neg	Neg	Pos
Riboflavin	7.5	Neg	Neg	Pos
Sodium azide	1,000	Neg	Neg	Pos
Sodium chloride	1,000	Neg	Neg	Pos
Sodium fluoride	1,000	Neg	Neg	Pos
Sodium phosphate	300	Neg	Neg	Pos
Urea	6,000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos

pH Interference Study:

pH 3 to pH 11 was tested for interference with the assay. Each pH level was split into three portions each and either left un-spiked or spiked to a fentanyl concentration of either 0.5 or 1.5 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No pH interference was observed.

pH	Spiked Fentanyl Concentration		
	0 ng/mL	0.5 ng/mL Control	1.5 ng/mL Control
pH 3	Neg	Neg	Pos
pH 4	Neg	Neg	Pos
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Specific Gravity: Samples ranging in specific gravity from 1.000 to 1.030 were split into three portions each and either left un-spiked or spiked to a fentanyl concentration of either 0.5 ng/mL or 1.5 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No interference was observed.

Specific Gravity	Spiked Fentanyl Concentration		
	0 ng/mL	0.5 ng/mL Control	1.5 ng/mL Control
1.000	Neg	Neg	Pos
1.003	Neg	Neg	Pos
1.005	Neg	Neg	Pos
1.008	Neg	Neg	Pos
1.010	Neg	Neg	Pos
1.011	Neg	Neg	Pos
1.013	Neg	Neg	Pos
1.015	Neg	Neg	Pos
1.018	Neg	Neg	Pos
1.020	Neg	Neg	Pos
1.022	Neg	Neg	Pos
1.023	Neg	Neg	Pos
1.025	Neg	Neg	Pos
1.030	Neg	Neg	Pos

Additional Information

For more detailed information on the AU 8 series and DxC AU Systems, refer to the appropriate system manual.

Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained, which is caused by the performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

Registered trademarks are the property of their respective owners.

Shipping Damage

Please notify your Beckman Coulter Clinical Support Center if this product is received damaged.

Bibliography:

- Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
- Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **23**(82):7920-7970 (2017).
- Ikeda R., Pelton C. Diversion programs for impaired physicians. *West J Med*, **152**: 617-621 (1990).
- Poklis, A., Fentanyl: a review for clinical and analytical toxicologists. *Clinical Toxicology*, **33**:439-447 (1995).
- Jaffe J.H., Martin W.R., Opioid analgesics and antagonists. In: Pharmacological Basis of Therapeutics, 7th Ed. Gilman AG, Goodman LS, Rall TW, Murad F, eds. New York: MacMillan, **517** (1985).
- Ellenhorn, M.D., Schonwald, S., Ordog, G., and J. Wasserberger, Fentanyl, in: Ellenhorn's Medical Toxicology, Williams & Wilkins, Baltimore, Philadelphia, London, Paris, Bangkok, Buenos Aires, Hong Kong, Munich, Sydney, Tokyo, Wrocław, 416-420 (1997).
- Atluri S., Sudarshan G., and L. Manchikanti, Assessment of the trends in medical use and misuse of opioid analgesics from 2004 to 2011. *Pain Physician*, **17**, E119-E128 (2014).
- McClain DA, Hug CC Jr., Intravenous fentanyl kinetics. *Clin Pharmacol Ther* **28**:106-114 (1980).
- Hug C.C., Murphy M.R., Anesthesiology, **55** (1981) 369. T. Tateishi, A.J.J. Wood, F.P. Guengerich and M. Wood, *Biochem. Pharmacol.*, **50** (1995) 1921.

Bibliography, continued:

10. Baselt R.C., Disposition of Toxic Drugs and Chemicals in Man, 10th edition. Biomedical Publications: Seal Beach, California; 846–849 (2014).
11. Baum R.M., New variety of street drugs poses growing problem. *Chem. Eng News* Sep **9**:7-16 (1985).
12. Suzuki S., Inoue, T., New abused drug “China White”. *Forensic Toxicol. News* **5**:5-12 (1987).
13. Heagy J.A., Identification of 4-propionoxy-4-phenyl-N-methylpiperidine. *Microgram* **15**: 181-186 (1982).
14. Poklis, J., Poklis, A., Wolf, C., Hathaway, C., Arbefeveille, E., Chrostowski, L., Devers, K., Hair, L., Mainland, M., Merves, M., and J. Pearson, *Journal of Analytical Toxicology*, **40**:703–708 (2016).
15. Backberg, M., Beck O., Jonsson K.H., Helander A., Opioid intoxications involving butyryl fentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. *Clinical toxicology*, **53**:609–617 (2015).
16. EMCDDA. European Drug Report 2014: Trends and developments 2014. Available at: http://www.emcdda.europa.eu/attachements.cfm/att_228272_EN_TDAT14001ENN.pdf.
17. EMCDDA. (2015) New psychoactive substances in Europe. An update from the EU Early Warning System (March 2015). Available at: http://www.emcdda.europa.eu/attachements.cfm/att_235958_EN_TD0415135ENN.pdf.
18. Helander A., Backberg, M., Hulten, P., Al-Saffer Y., and O. Beck, Detection of new psychoactive substance use among emergency room patients: results from Swedish STRIDA project. *Forensic Science International*, **243**:23–29(2014).
19. Rubenstein K.E., Schneider R.S., Ullman E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**: 846 (1972).
20. Sodium Azide. National Institute for Occupational Safety (NIOSH) Pocket Guide to Chemical Hazards Third Printing, September 2007. Available online at: <https://www.cdc.gov/niosh/npg/default.html>
21. Thevis, M., Geyer, H., Bahr, D., and W. Schänzer. Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. *Eur. J. Mass Spectrom.* **11**:419–427 (2005).
22. Gonzales, E., Ng, G., Pesce, A., West, C., West, R., Mikel, C., Latyshev, S., and P. Almazan. Stability of pain-related medications, metabolites, and illicit substances in urine. *Clinica Chimica Acta* **416**:80–85 (2013).
23. Concheiro, M., Chesser, R., Pardi, J., and G. Cooper. Postmortem Toxicology of New Synthetic Opioids. *Front Pharmacol* (2018) **9**:1-18.

Symbols

LZI uses the symbols and signs listed on the symbol glossary on the website. Visit www.lin-zhi.com/symbol-glossary for detailed information.

| Additions, deletions, or changes are indicated by a change bar in the margin.

For instructions for use (including translations) please visit:

https://www.lin-zhi.com/bci_applications/



Manufacturer:

Lin-Zhi International, Inc.

2945 Oakmead Village Court

Santa Clara, CA 95051

USA

Tel: (408) 970-8811

Fax: (408) 970-9030

www.lin-zhi.com

© July 2025 Rev. 0