LZI Fentanyl Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0310 (100/37.5 mL R_1/R_2 Kit)
0311 (1000/375 mL R_1/R_2 Kit) 2°C 8°C

Lin-Zhi International, Inc. *Outside USA Only*

Intended Use

The LZI Fentanyl Enzyme Immunoassay is an in vitro diagnostic test intended for the qualitative and semi-quantitative determination of norfentanyl in human urine at the cutoff value of 5 ng/mL when calibrated against norfentanyl. The assay is designed for screening with the Beckman Coulter AU680 and AU480 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 (1). 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 (2, 3) and cases of fentanyl abuse via intravenous injection, inhalation, oral, or nasal applications have been previously reported (4). 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 (5).

Due to its short elimination half-life and approximately 90% metabolism, fentanyl is difficult to detect in urine (6). Fentanyl undergoes extensive hepatic biotransformation to metabolites coming from hydrolysis, N-dealkylation, or hydroxylation reactions (7). In an intravenous dose of fentanyl, up to 85% is excreted in urine over a 3- to 4-day period with 0.4-6% eliminated as unchanged fentanyl and 26-55% eliminated as the norfentanyl metabolite (8).

Fentanyl analogs also have high potency analgesic activities. Numerous reports have been published with modified fentanyl-related compounds abused as designer drugs (9-11).

Other recently available fentanyl analogs associated with abuse and severe intoxication include butyryl fentanyl and 4-fluorobutyryl fentanyl (12-16).

Assay Principle

The LZI Fentanyl 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 (17). 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 (R_1): 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 (R_2): 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.*

NORFENTANYL Calibrator	REF
Cutoff Calibrator: Contains 5 ng/mL norfentanyl	0313

NORFENTANYL Controls	REF
Level 1 Control: Contains 3.75 ng/mL norfentanyl	0317
Level 2 Control: Contains 6.25 ng/mL norfentanyl	0318

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 (18).*
- *Do not use the reagents beyond their expiration dates.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use. See the 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 2-8ºC for up to four weeks (19) or at room temperature for up to four weeks (19, 20). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown norfentanyl samples in urine are stable at -20ºC for up to six months (21).

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 (18-25ºC) 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 AU680 and AU480.

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU680 and AU480 analyzers include a 15 µL sample, 120 µL of antibody reagent (R_1), 45 µL of enzyme conjugate reagent (R_2), 10 µL dilution following addition of R_2 in 37ºC incubation temperature, 14-19 reading points, and 340 nm primary wavelength. Additional washing steps are required, please refer to the specific parameters used for each analyzer before performing the assay.

For qualitative analysis use the 5 ng/mL as the cutoff calibrator. 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: 3.75 ng/mL and 6.25 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 LZI technical support 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 5 ng/mL of norfentanyl, 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 from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS or LC/MS or (2) permitting laboratories to establish quality control procedures. When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of norfentanyl in the sample may then be estimated from the calibration curve. Results below the respective cutoff are considered negatives. Results equal to or above the respective cutoff are considered preliminary positives.

Limitations

1. *Boric Acid at 1% w/v may cause false negative results. Boric Acid is not recommended as a preservative for urine.*
2. *Dextromethorphan may cause false positive results at concentrations greater than 25,000 ng/mL.*
3. *A preliminary positive result from this assay indicates only the presence of norfentanyl and does not necessarily correlate with the extent of physiological and psychological effects (e.g., intoxication). This test is not intended for quantifying the individual analytes in samples.*
4. *A negative result does not necessarily mean a person did not abuse drugs.*
5. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
6. *Preliminary positive results should be confirmed by other affirmative, analytical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
7. *The test is designed for use with human urine only.*
8. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with Beckman Coulter AU680 and AU480 (master) automated chemistry analyzers. Precision and accuracy sections show data from AU680 and AU480.

Precision on Beckman Coulter AU680:

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

Cutoff		Within Run(N=22)		Run-to-Run(N=88)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0%	22	22 Neg	88	88 Neg
1.25 ng/mL	25%	22	22 Neg	88	88 Neg
2.5 ng/mL	50%	22	22 Neg	88	88 Neg
3.75 ng/mL	75%	22	22 Neg	88	88 Neg
5 ng/mL	100%	22	20 Neg/ 2 Pos	88	62 Neg/ 26 Pos
6.25 ng/mL	125%	22	22 Pos	88	88 Pos
7.5 ng/mL	150%	22	22 Pos	88	88 Pos
8.75 ng/mL	175%	22	22 Pos	88	88 Pos
10 ng/mL	200%	22	22 Pos	88	88 Pos

Semi-Quantitative analysis: The following concentrations were evaluated. Typical semi-quantitative results (ng/mL) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.0	0.2	N/A	0.0	0.2	N/A
1.25 ng/mL	1.4	0.2	13.6%	1.4	0.2	15.8%
2.5 ng/mL	2.6	0.2	6.1%	2.6	0.2	8.4%
3.75 ng/mL	3.8	0.2	5.3%	3.8	0.2	6.3%
5 ng/mL	5.2	0.2	3.5%	5.2	0.2	4.6%
6.25 ng/mL	6.4	0.2	3.6%	6.4	0.3	4.9%
7.5 ng/mL	8.0	0.3	3.3%	8.0	0.3	3.7%
8.75 ng/mL	9.2	0.2	2.0%	9.2	0.3	2.8%
10 ng/mL	10.2	0.4	3.8%	10.2	0.5	4.4%

Precision on Beckman Coulter AU480:

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

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.3	0.7	N/A	0.3	0.9	N/A
3.75 ng/mL	55.8	3.0	5.4%	55.8	3.6	6.5%
5 ng/mL	77.6	4.1	5.3%	77.6	4.4	5.7%
6.25 ng/mL	96.2	3.2	3.3%	96.2	4.0	4.2%

Semi-Quantitative analysis: The following concentrations were evaluated. Typical semi-quantitative results (ng/mL) are as follows:

Concentration	Within Run (N=22)			Total Precision (N=88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.4	0.2	N/A	0.4	0.2	N/A
3.75 ng/mL	4.0	0.2	4.0%	4.0	0.2	5.6%
5 ng/mL	5.4	0.2	3.3%	5.4	0.2	4.6%
6.25 ng/mL	6.5	0.2	2.7%	6.5	0.2	3.4%

Accuracy on Beckman Coulter AU680: One-hundred and one (101) unaltered clinical urine specimens were tested with the LZI Fentanyl Enzyme Immunoassay and confirmed by LC/MS. Specimens having a norfentanyl concentration greater than 5 ng/mL by LC/MS are defined as positive, and specimens with norfentanyl concentrations below 5 ng/mL by LC/MS are defined as negative in the table below. Near cutoff samples are defined as ±50% of the cutoff value. The correlation results are summarized as follows:

Qualitative Accuracy Study:

Cutoff	Neg	< 50% of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	1*	6**	8	41	100.0%
Negative	21	19	5	0	0	86.5%

The following table summarizes the result for the discrepant samples:

Sample #	Norfentanyl LC/MS (ng/mL)	LC/MS	LZI EIA (mAU)	Cutoff Value (mAU)	LZI EIA
38*	1.5	Neg	76.7	68.5	Pos
44**	3.0	Neg	82.2	64.1	Pos
46**	3.3	Neg	121.9	68.5	Pos
47**	3.5	Neg	151.4	64.0	Pos
48**	3.8	Neg	192.4	64.0	Pos
50**	4.2	Neg	115.8	63.1	Pos
52**	4.6	Neg	162.2	68.5	Pos

* Values are discrepant between -50% of the cutoff to the cutoff concentration (2.5 ng/mL – 4.9 ng/mL)
** Values are discrepant between cutoff to +50% of the cutoff concentration (5 ng/mL – 7.4 ng/mL)

Semi-Quantitative Accuracy Study:

Cutoff	Neg	< 50% of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	0	5*	6	45	96.2%
Negative	20	17	6	2**	0	89.6%

The following table summarizes the result for the discrepant samples:

Sample #	Norfentanyl LC/MS (ng/mL)	LC/MS	LZI EIA (ng/mL)	LZI EIA
41*	0	Neg	6.0	Pos
44*	1.1	Neg	6.1	Pos
45*	0.3	Neg	14.0	Pos
46*	0.2	Neg	18.5	Pos
48*	0.7	Neg	9.6	Pos
50**	2.7	Neg	4.4	Neg
52**	1.1	Neg	4.6	Neg

* Values are discrepant between -50% of the cutoff to the cutoff concentration (2.5 ng/mL – 4.9 ng/mL)
** Values are discrepant between cutoff to +50% of the cutoff concentration (5 ng/mL – 7.4 ng/mL)

Accuracy on Beckman Coulter AU480: One-hundred (100) unaltered clinical urine specimens were tested with the LZI Fentanyl Enzyme Immunoassay and confirmed by LC/MS. Specimens having a norfentanyl concentration equal to or greater than 5 ng/mL by LC/MS are defined as positive, and specimens with norfentanyl concentrations below 5 ng/mL by LC/MS are defined as negative in the table below. Near cutoff samples are defined as ±50% of the cutoff value. The correlation results are summarized as follows:

Qualitative Accuracy Study:

Cutoff	Neg	< 50% of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	1*	8**	10	40	100.0%
Negative	20	19	2	0	0	82.0%

Qualitative Accuracy Study, continued:
The following table summarizes the result for the discrepant samples:

Sample #	Norfentanyl LC/MS (ng/mL)	LC/MS	LZI EIA (mAU)	Cutoff Value (mAU)	LZI EIA
37*	1.5	Neg	68.2	67.6	Pos
41**	2.7	Neg	108.7	67.6	Pos
43**	3.0	Neg	186.8	67.6	Pos
44**	3.0	Neg	92.3	67.6	Pos
45**	3.3	Neg	107.6	67.6	Pos
46**	3.5	Neg	160.1	67.6	Pos
47**	3.8	Neg	188.6	67.6	Pos
48**	3.8	Neg	102.4	67.6	Pos
49**	4.2	Neg	235.6	67.6	Pos

* Values are discrepant between negative and 50% below cutoff concentration (0.1 – 2.4 ng/mL)
** Values are discrepant between 50% below cutoff and cutoff concentration (2.5 – 4.9 ng/mL)

Semi-Quantitative Accuracy Study:

Cutoff	Neg	< 50% of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agree-ment
Positive	0	1*	8**	10	40	100.0%
Negative	20	19	2	0	0	82.0%

The following table summarizes the result for the discrepant samples:

Sample #	Norfentanyl LC/MS (ng/mL)	LC/MS	LZI EIA (ng/mL)	LZI EIA
37*	1.5	Neg	5.1	Pos
41**	2.7	Neg	7.8	Pos
43**	3.0	Neg	15.8	Pos
44**	3.0	Neg	6.8	Pos
45**	3.3	Neg	8.3	Pos
46**	3.5	Neg	13.5	Pos
47**	3.8	Neg	17.5	Pos
48**	3.8	Neg	7.7	Pos

* Values are discrepant between negative and 50% below cutoff concentration (0.1 – 2.4 ng/mL)
** Values are discrepant between 50% below cutoff and cutoff concentration (2.5 – 4.9 ng/mL)

Specificity on Beckman Coulter AU680: 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	3.2	156.25%	Pos
Norfentanyl	5	100.00%	Pos

Structurally Related Compounds:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity	Result
4-Fluoro-isobutyryl Fentanyl	35	14.29%	Pos
9-HydroxyRisperidone	100000	0.01%	Neg
Acetyl Fentanyl	7	71.43%	Pos
Acetyl Norfentanyl	100	5.00%	Pos
Acryl Fentanyl	3.5	142.86%	Pos
Alfentanil	100000	0.01%	Neg
Butyryl Fentanyl	3.5	142.86%	Pos
Butyryl Norfentanyl	35	14.29%	Pos
Carfentanil Oxalate	100000	0.01%	Neg
Cis-d, I 3-Methylfentanyl	8.5	58.82%	Pos
Cyclopropyl Norfentanyl	20	25.00%	Pos
Despropionylfentanyl (4-ANPP)	100000	0.01%	Neg
Furanyl Fentanyl	6	81.97%	Pos
Furanyl Norfentanyl	180	2.78%	Pos
(±)-β-Hydroxythiofentanyl	5	100.00%	Pos
Isobutyryl Fentanyl	20	25.00%	Pos
Isobutyryl Norfentanyl	400	1.25%	Pos
Labetalol Hydrochloride	100000	0.01%	Neg
Methoxyacetyl Fentanyl	3.5	142.86%	Pos
MT-45	100000	0.01%	Neg
N-benzyl Furanyl Norfentanyl	12	41.67%	Pos
N-benzyl para-fluoro Norfentanyl	4.2	119.05%	Pos
Norcarfentanil Oxalate	100000	0.01%	Neg
Ocfentanil	3.5	142.86%	Pos

Structurally Related Compounds, continued:

Compound	Concentration Tested (ng/mL)	% Cross-Reactivity	Result
Para-fluorobutyryl Fentanyl (P-FBF)	5.5	90.91%	Pos
para-Fluorofentanyl	3.1	163.93%	Pos
Remifentanil	100000	0.01%	Neg
Risperidone	100000	0.01%	Neg
Sufentanil	100000	0.01%	Neg
Thienyl Fentanyl	3.5	142.86%	Pos
Thiofentanyl	3.2	156.25%	Pos
Trans-d, I 3-Methylfentanyl	6	83.33%	Pos
Trazodone	100000	0.01%	Neg
U-47700	100000	0.01%	Neg
Valeryl Fentanyl	95	5.26%	Pos
ω-1-Hydroxy Fentanyl	320	1.56%	Pos

Structurally Unrelated Compounds:

Compound	Spiked [ ] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
(1S,2S)-(+)Pseudoephedrine	100000	ND	Neg	Pos
6-Acetylmorphine	10000	ND	Neg	Pos
Acetaminophen	100000	ND	Neg	Pos
Acetylsalicylic Acid	100000	ND	Neg	Pos
Amitriptyline	100000	ND	Neg	Pos
Amlodipine Besylate	100000	ND	Neg	Pos
Amoxicillin	100000	ND	Neg	Pos
Atorvastatin	20000	ND	Neg	Pos
Benzoylecgonine	100000	ND	Neg	Pos
Buprenorphine	100000	ND	Neg	Pos
Bupropion	100000	ND	Neg	Pos
Caffeine	100000	ND	Neg	Pos
Carbamazepine	100000	ND	Neg	Pos
Cetirizine	100000	ND	Neg	Pos
Chlorpheniramine	100000	ND	Neg	Pos
Chlorpromazine	100000	ND	Neg	Pos
Clomipramine	100000	ND	Neg	Pos
Codeine	100000	ND	Neg	Pos
d-Amphetamine	100000	ND	Neg	Pos
Desipramine	100000	ND	Neg	Pos
Dextromethorphan	40000	Pos	Pos	Pos
Diphenhydramine	100000	ND	Neg	Pos
d-Methamphetamine	100000	ND	Neg	Pos
Duloxetine	100000	ND	Neg	Pos
Fluoxetine	100000	ND	Neg	Pos
Fluphenazine	100000	ND	Neg	Pos
Gabapentin	100000	ND	Neg	Pos
Hydrocodone	100000	ND	Neg	Pos
Hydromorphone	100000	ND	Neg	Pos
Ibuprofen	100000	ND	Neg	Pos
Imipramine	100000	ND	Neg	Pos
Lisinopril	100000	ND	Neg	Pos
Loratadine	100000	ND	Neg	Pos
Losartan	10000	ND	Neg	Pos
l-Thyroxine	10000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100000	ND	Neg	Pos
MDEA	100000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100000	ND	Neg	Pos
Meperidine	100000	ND	Neg	Pos
Metformin	100000	ND	Neg	Pos
Methadone	100000	ND	Neg	Pos
Metoprolol	100000	ND	Neg	Pos
Morphine	100000	ND	Neg	Pos
Nicotine	100000	ND	Neg	Pos
Nortriptyline	100000	ND	Neg	Pos
Omeprazole	100000	ND	Neg	Pos
Oxazepam	100000	ND	Neg	Pos
Oxycodone	100000	ND	Neg	Pos
Oxymorphone	100000	ND	Neg	Pos
Phencyclidine (PCP)	100000	ND	Neg	Pos
Phenobarbital	100000	ND	Neg	Pos
Quetiapine	100000	ND	Neg	Pos
Ranitidine	100000	ND	Neg	Pos
Salbutamol (Albuterol)	100000	ND	Neg	Pos
Sertraline	100000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	100000	ND	Neg	Pos
Tramadol	100000	ND	Neg	Pos
Zolpidem	10000	ND	Neg	Pos

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

Structurally Unrelated Compounds, continued:
The following structurally unrelated compound which showed interference at ±25% of cutoff concentrations was then spiked into pooled negative human urine at ±50% of cutoff concentrations (2.5 ng/mL and 7.5 ng/mL) for the assay. Interference was still observed with dextromethorphan. Results are summarized in the following table:

Compound	Spiked [ ] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	2.5 ng/mL	7.5 ng/mL
Dextromethorphan	40000	Pos	Pos	Pos

Endogenous and Preservative Compound Interference Study on Beckman Coulter AU680:
The following endogenous compounds were spiked into pooled negative human urine and the two levels of controls (3.75 ng/mL and 6.25 ng/mL) for the assay. The spiked solution was evaluated against cutoff calibrator. Interference was observed with Boric Acid. No other major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 5 ng/mL. Results are summarized in the following table:

Endogenous or Preservative Substance	Spiked [ ] (mg/dL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL Control	6.25 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride (CaCl2)	300	Neg	Neg	Pos
Citric Acid (pH 3)	800	Neg	Neg	Neg
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Neg
Riboflavin	7.5	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	6000	Neg	Neg	Pos

The following endogenous compounds which showed interference at ±25% of cutoff concentrations were then spiked into negative urine and at ±50% of cutoff concentrations (2.5 ng/mL and 7.5 ng/mL) for the assay. Interference was still observed with Boric Acid at 1% w/v. Results are summarized in the following table:

Endogenous or Preservative Substance	Spiked [ ] (mg/dL)	Spiked Norfentanyl Concentration		
		0 ng/mL	2.5 ng/mL	7.5 ng/mL
Boric Acid	1000	Neg	Neg	Neg
Citric Acid (pH 3)	800	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos

pH Interference Study on Beckman Coulter AU680: Negative urine and urine spiked with analyte to the two levels of controls (3.75 ng/mL and 6.25 ng/mL) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were evaluated against the cutoff calibrator. No major interference with these pH levels was observed as all pH adjusted levels gave correct corresponding preliminary positive/negative results against the cutoff value of 5 ng/mL. Results are summarized in the following table:

pH	Spiked Norfentanyl Concentration		
	0 ng/mL	3.75 ng/mL Control	6.25 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 on Beckman Coulter AU680: Samples ranging in specific gravity from 1.003 to 1.028 were split into three portions each and either left un-spiked or further spiked to a final norfentanyl concentration of either 3.75 ng/mL or 6.25 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No interference was observed.

Open-Vial Reagent and Calibrator/Control Stability on Beckman Coulter AU680: Real-time data for open-vial reagent and calibrator/control stability studies at Cold Temperature (2-8ºC) have been carried out up to Day 736. Results from open-vial studies indicate that degradation is minimal up to Day 736, and, based on the real-time data, suggests an open-vial stability of up to 24 months. Open-vial reagents and calibrators/controls should be stored at 2-8ºC for maximum shelf life.

Closed-Vial Calibrator/Control Stability on Beckman Coulter AU680: Real-time data for closed-vial calibrator/control stability studies at Cold Temperature (2-8ºC) have been carried out up to Day 736. Results from closed-vial studies indicate that degradation is minimal at Cold Temperature (2-8ºC) up to Day 736 in comparison to Day 1. Closed-vial calibrators/controls should be stored at 2-8ºC for maximum shelf life.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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.
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