

URINE FENTANYL

The LZI Fentanyl Enzyme Immunoassay is intended for the qualitative determination of norfentanyl in human urine.

Endogenous and Preservative Compound Interference Study:

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 Substance	Spiked [] (mg/dL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL	6.25 ng/mL
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride (CaCl ₂)	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 Substance	Spiked [] (mg/dL)	Spiked Fentanyl Conc.		
		0 ng/mL	3.75 ng/mL	6.25 ng/mL
Boric Acid	1000	Neg	Neg	Neg
Citric Acid (pH 3)	800	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos

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	3.2	156.25 %	Positive
Norfentanyl	5	100.00 %	Positive

Structurally Related Compounds:

Compound	Concentration Tested (ng/mL)	% Cross Reactivity	Result
4-Fluoro-isobutyryl Fentanyl	35	14.29 %	Pos
9-HydroxyRisperidone	100,000	0.01 %	Neg
Acetyl Fentanyl	7	71.43 %	Pos
Acetyl Norfentanyl	100	5.00 %	Pos
Acryl Fentanyl	3.5	142.86 %	Pos
Alfentanil	100,000	0.01 %	Neg
Butyryl Fentanyl	3.5	142.86 %	Pos
Butyryl Norfentanyl	35	14.29 %	Pos
Carfentanil Oxalate	100,000	0.01 %	Neg
Cis-d, l 3-Methylfentanyl	8.5	58.82 %	Pos
Cyclopropyl Norfentanyl	20	25.00 %	Pos
Despropionylfentanyl (4-ANPP)	100,000	0.01 %	Neg
Furanyl Fentanyl	6	81.97 %	Pos
Furanyl Norfentanyl	180	2.78 %	Pos
(±)-β-Hydroxythiofentanyl	5	100.00 %	Po
Isobutyryl Fentanyl	20	25.00 %	Pos
Isobutyryl Norfentanyl	400	1.25 %	Pos
Labetalol Hydrochloride	100,000	0.01 %	Neg
Methoxyacetyl Fentanyl	3.5	142.86 %	Pos
MT-45	100,000	0.01 %	Neg
N-benzyl Furanyl Norfentanyl	12	41.67 %	Pos
N-benzyl para-fluoro Norfentanyl	4.2	119.05 %	Pos
Norcarfentanil Oxalate	100,000	0.01 %	Neg

Ocfentanil	3.5	142.86 %	Pos
Para-fluorobutyryl Fentanyl (P-FBF)	5.5	90.91 %	Pos
para-Fluorofentanyl	3.1	163.93 %	Pos
Remifentanil	100,000	0.01 %	Neg
Risperidone	100,000	0.01 %	Neg
Sufentanil	100,000	0.01 %	Neg
Thienyl Fentanyl	3.5	142.86 %	Pos
Thiofentanyl	3.2	156.25 %	Pos
Trans-d, l 3-Methylfentanyl	6	83.33 %	Pos
Trazodone	100,000	0.01 %	Neg
U-47700	100,000	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	6.25 ng/mL
(1S,2S)-(+)-Pseudoephedrine	100,000	ND	Neg	Pos
6-Acetylmorphine	10,000	ND	Neg	Pos
Acetaminophen	100,000	ND	Neg	Pos
Acetylsalicylic Acid	100,000	ND	Neg	Pos
Amitriptyline	100,000	ND	Neg	Pos
Amlodipine Besylate	100,000	ND	Neg	Pos
Amoxicillin	100,000	ND	Neg	Pos
Atorvastatin	20,000	ND	Neg	Pos
Benzoylcegonine	100,000	ND	Neg	Pos
Buprenorphine	100,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
d-Amphetamine	100,000	ND	Neg	Pos
Desipramine	100,000	ND	Neg	Pos
Dextromethorphan	40,000	Pos	Pos	Pos
Diphenhydramine	100,000	ND	Neg	Pos
d-Methamphetamine	100,000	ND	Neg	Pos
Duloxetine	100,000	ND	Neg	Pos
Fluoxetine	100,000	ND	Neg	Pos
Fluphenazine	100,000	ND	Neg	Pos
Gabapentin	100,000	ND	Neg	Pos
Hydrocodone	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Neg	Pos
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos

Loratadine	100,000	ND	Neg	Pos
Losartan	10,000	ND	Neg	Pos
l-Thyroxine	10,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100,000	ND	Neg	Pos
Meperidine	100,000	ND	Neg	Pos
Metformin	100,000	ND	Neg	Pos
Methadone	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
Morphine	100,000	ND	Neg	Pos
Nicotine	100,000	ND	Neg	Pos
Nortriptyline	100,000	ND	Neg	Pos
Omeprazole	100,000	ND	Neg	Pos
Oxazepam	100,000	ND	Neg	Pos
Oxycodone	100,000	ND	Neg	Pos
Oxymorphone	100,000	ND	Neg	Pos
Phencyclidine (PCP)	100,000	ND	Neg	Pos
Phenobarbital	100,000	ND	Neg	Pos
Quetiapine	100,000	ND	Neg	Pos
Ranitidine	100,000	ND	Neg	Pos
Salbutamol (Albuterol)	100,000	ND	Neg	Pos
Sertraline	100,000	ND	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	100,000	ND	Neg	Pos
Tramadol	100,000	ND	Neg	Pos
Zolpidem	10,000	ND	Neg	Pos

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

The following structurally unrelated compound which showed interference at $\pm 25\%$ of cutoff concentrations was then spiked into pooled negative human urine at $\pm 50\%$ 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:

Endogenous Substance	Spiked [] (ng/mL)	Spiked Norfentanyl Concentration		
		0 ng/mL	3.75 ng/mL	6.25 ng/mL
Dextromethorphan	40,000	Pos	Pos	Pos

Accuracy

One-hundred and one (101) unaltered clinical urine specimens were tested with the LZI Fentanyl (Q) 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 $\pm 50\%$ of the cutoff value. The correlation results are summarized as follows:

	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
5 ng/mL Cutoff						
Positive	0	1*	6**	8	41	100.0 %
Negative	21	19	5	0	0	86.5 %

CAMC laboratories use a 5 ng/mL cutoff for the fentanyl assay.

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