ARK Urine Fentanyl II Assay

Analytical Specificity

All compounds tested were added to drug-free, negative human urine.

The cross-reactivity of the following metabolites and structural analogs of fentanyl was evaluated by spiking these compounds into drug-free, negative human urine and evaluated by dose-response to determine the approximate equivalence to the 1.0 ng/mL fentanyl cutoff. These concentrations were used to determine the percent cross-reactivity according to the formula:
% Cross-reactivity = (Cutoff concentration / Concentration approximately equivalent to the 1.0 ng/mL cutoff) X 100

For the compounds Alfentanil and Remifentanil that did not produce a positive result, the highest concentration tested was used to calculate percent cross-reactivity.

Cross-reactivity

For the major metabolite, norfentanyl, the lowest concentration capable of producing a positive result is shown below.

Norfentanyl (Major Metabolite)

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
Norfentanyl	15	7

Other Metabolites and Structural Analogs of Fentanyl

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
Acetyl fentanyl	1.1	90.91
Isobutyryl fentanyl	1.1	90.91
ω-1-Hydroxyfentanyl	1.2	83.33
Acrylfentanyl	1.3	76.92
Butyryl fentanyl	1.4	71.43
Furanyl fentanyl	1.5	66.67
Para-fluoro fentanyl	1.5	66.67
Ocfentanil	1.6	62.50
4-Fluoro-isobutyryl fentanyl	1.9	52.63
Para-fluorobutyryl fentanyl (p-FBF)	1.9	52.63

Compound	Concentration Approximately Equivalent to the Cutoff (ng/mL)	Cross-reactivity (%)
Valeryl fentanyl	2.3	43.48
β-hydroxyfentanyl	9.5	10.53
Acetyl norfentanyl	12.1	8.26
(±) β-hydroxythiofentanyl	32.7	3.06
(±)-3-cis-methyl fentanyl	144.1	0.69
Carfentanil	448.2	0.22
Despropionyl fentanyl (4-ANPP)	471.8	0.21
Sufentanil	2,362	0.04
Remifentanil	10,000	<0.01
Norcarfentanil	38,196	0.003
Alfentanil	100,000	<0.001

The following **opioids, structurally similar compounds, and functional analogs** were negative at the concentrations tested with the ARK Fentanyl II Assay.

Compound	Concentration Tested (ug/mL)	Compound	Concentration Tested (ug/mL)
6-Acetyl morphine	100	Naltrexone	100
Buprenorphine	100	Norbuprenorphine	100
Buprenorphine glucuronide	100	Norcodeine	100
Codeine	100	Normeperidine	100
Dextromethorphan	100	Normorphine	100
Dihydrocodeine	100	Noroxycodone	100
EDDP	100	Oxycodone	100
EMDP	100	Oxymorphone	100
Heroin	100	Pentazocine (Talwin)	100
Hydrocodone	100	Pipamperone	90
Hydromorphone	100	Quinine	100
9-Hydroxyrisperidone	100	Quinidine	100
Labetalol	100	Risperidone	100
Levorphanol	100	Tapentadol	100
m-Chlorophenylpiperazine (m-CPP)	100	Thioridazine	100
Meperidine	100	Tilidint	100
Methadone	100	Tramadol	100
Morphine	100	Tramadol-O-Desmethyl	100
Morphine-3-glucuronide	100	Tramadol-N-Desmethyl	100
Naloxone	100	Trazodone	100

Interence- Structurally Unrelated Compounds

High concentrations of the following structurally unrelated compounds were added into fentanyl-spiked urine (± 50% of the cutoff concentration). The substances listed below did not yield a false result relative to the cutoff.

Compound	Concentration Tested (ug/mL)	0.5 ng/mL (50% Cutoff)	1.5 ng/mL (+50% Cutoff)
Acetaminophen	500	Negative	Positive
Acetylsalicylic Acid	1000	Negative	Positive
Albuterol	100	Negative	Positive
Amitriptyline	100	Negative	Positive
Amobarbital	100	Negative	Positive
Amphetamine	100	Negative	Positive
Benzoylecgonine	100	Negative	Positive
Bupropion	100	Negative	Positive
Caffeine	100	Negative	Positive
Carbamazepine	100	Negative	Positive
Chlorpromazine	100	Negative	Positive
Clomipramine	100	Negative	Positive
Cyclobenzaprine	100	Negative	Positive
Desipramine	100	Negative	Positive
Doxepin	100	Negative	Positive
Ecgonine	100	Negative	Positive
Ephedrine	100	Negative	Positive
Fluoxetine	100	Negative	Positive
Fluphenazine	100	Negative	Positive
Ibuprofen	500	Negative	Positive
Imipramine	100	Negative	Positive
Ketamine	100	Negative	Positive
Lidocaine	100	Negative	Positive
Maprotiline	100	Negative	Positive
Methapyrilene	100	Negative	Positive
Methaqualone	100	Negative	Positive
Metronidazole	300	Negative	Positive
Nicotine	100	Negative	Positive
Norketamine	100	Negative	Positive
Nortriptyline	60	Negative	Positive
Oxazepam	100	Negative	Positive
Phencyclidine	100	Negative	Positive
Phenobarbital	100	Negative	Positive
Propoxyphene	100	Negative	Positive
Ranitidine	100	Negative	Positive
Secobarbital	100	Negative	Positive
Valproic Acid	250	Negative	Positive
Venlafaxine	100	Negative	Positive

Interference- Endogenous Substance
High concentrations of the following endogenous substances were added onto fentanyl-spiked urine (± 50% of the cutoff concentration). No interference was observed when tested with the ARK Fentanyl II Assay.

Compound	Concentration Tested (mg/dL)	Negative	Positive
Acetone	1000	Negative	Positive
Ascorbic Acid	560	Negative	Positive
Bilirubin	2	Negative	Positive
Creatinine	500	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
Gamma Globulin	500	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	500	Negative	Positive
Human Albumin	500	Negative	Positive
Oxalic Acid	100	Negative	Positive
Riboflavin	7.5	Negative	Positive
Sodium Chloride	4000	Negative	Positive
Urea	2000	Negative	Positive

Interference – Boric Acid

One percent (1%) w/v of boric acid was added into fentanyl-spiked urine (± 50% of the cutoff concentration). Results are provided in the table below.

Compound	Concentration Tested	0.5 ng/mL (-50% Cutoff)	1.5 ng/mL (+50% Cutoff)
Boric Acid	1% w/v	Negative	Negative

Interference- Specific Gravity and pH
Urine samples with specific gravity values from 1.002 to 1.030 and pH values ranging from 3.0 to 11.0 were testes in the presence of the two levels of fentanyl at ± 50% of the cutoff concentration. No interference was observed when tested with the ARK Fentanyl II Assay.