

LZI Fentanyl Enzyme Immunoassay

For Beckman Coulter, Inc.

REF C68809 (100/37.5 mL R₁/R₂ Kit)

2-8°C

IVD For In Vitro Diagnostic Use Only



Lin-Zhi International, Inc.

For Sales Outside USA (OUS) Only

Intended Use

The LZI Fentanyl Enzyme Immunoassay for Beckman Coulter, Inc. is 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 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 3- to 4- 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 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*

*Calibrators and Controls are sold separately or as a semi-quantitative set and contain negative human urine with sodium azide as a preservative.

| Qualitative Calibration | REF |
|--|--------|
| LZI Norfentanyl Qualitative Calibrator | C68810 |
| NFEN Cutoff Calibrator (5 ng/mL) | |

Calibrators and Controls, continued

| Semi-Quantitative Calibration | REF |
|--|--------|
| LZI Universal Negative Calibrator | C68807 |
| LZI Norfentanyl Semi-Quantitative Calibrator Set | C68811 |
| NFEN Low Calibrator (2.5 ng/mL) | |
| NFEN Cutoff Calibrator (5 ng/mL) | |
| NFEN Intermediate Calibrator (10 ng/mL) | |
| NFEN High Calibrator (20 ng/mL) | |

| Controls | REF |
|-----------------------------------|--------|
| LZI Norfentanyl Level 1 Control | C68821 |
| NFEN Level 1 Control (3.75 ng/mL) | |
| LZI Norfentanyl Level 2 Control | C68822 |
| NFEN Level 2 Control (6.25 ng/mL) | |

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.

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.

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 (19) 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 norfentanyl 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 AU680.

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. Additional washing steps are required, reference analyzer specific parameter sheet. For qualitative analysis, use the 5 ng/mL as the cutoff calibrator. The cutoff is normalized to 100. Positive samples are ≥ 100 and are flagged with a (P). For semi-quantitative analysis, use all five calibrators including the universal negative 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 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.

Qualitative: The cutoff calibrator, which contains 5 ng/mL of norfentanyl, is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (Δ mAU) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (Δ mAU) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, 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.

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 25,000 ng/mL.
- 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.
- A negative result does not necessarily mean a person did not abuse 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 AU680 automated chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from 5 calibrators. Typical results were measured in ng/mL.

| 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 % |

| 5 ng/mL Cutoff | | Within Run (N=22) | | Run-to-Run (N=88) | |
|----------------|-------------|-------------------|------------------|-------------------|-------------------|
| Concentration | % of Cutoff | # Samples | EIA Result | # Samples | EIA Result |
| 0 ng/mL | -100.0 % | 22 | 22 Neg | 88 | 88 Neg |
| 1.25 ng/mL | -75.0 % | 22 | 22 Neg | 88 | 88 Neg |
| 2.5 ng/mL | -50.0 % | 22 | 22 Neg | 88 | 88 Neg |
| 3.75 ng/mL | -25.0 % | 22 | 22 Neg | 88 | 88 Neg |
| 5 ng/mL | 100.0 % | 22 | 3 Neg/ 19 Pos | 88 | 24 Neg/ 64 Pos |
| 6.25 ng/mL | +25.0 % | 22 | 22 Pos | 88 | 88 Pos |
| 7.5 ng/mL | +50.0 % | 22 | 22 Pos | 88 | 88 Pos |
| 8.75 ng/mL | +75.0 % | 22 | 22 Pos | 88 | 88 Pos |
| 10 ng/mL | +100.0 % | 22 | 22 Pos | 88 | 88 Pos |

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

| Concentration | Within Run (N=22) | | | Total Precision (N=88) | | |
|---------------|-------------------|-----|--------|------------------------|-----|--------|
| | Mean | SD | % CV | Mean | SD | % CV |
| 0 ng/mL | -11.1 | 4.3 | N/A | -11.1 | 5.2 | N/A |
| 1.25 ng/mL | 12.2 | 3.2 | 26.9 % | 12.2 | 3.8 | 31.2 % |
| 2.5 ng/mL | 34.1 | 3.3 | 9.9 % | 34.1 | 4.1 | 12.0 % |
| 3.75 ng/mL | 52.3 | 3.9 | 7.6 % | 52.3 | 5.0 | 9.7 % |
| 5 ng/mL | 71.9 | 3.2 | 4.5 % | 71.9 | 4.5 | 6.3 % |
| 6.25 ng/mL | 92.5 | 3.4 | 3.6 % | 92.5 | 4.1 | 4.4 % |
| 7.5 ng/mL | 115.2 | 4.1 | 3.6 % | 115.2 | 4.7 | 4.1 % |
| 8.75 ng/mL | 132.6 | 3.7 | 2.8 % | 132.6 | 4.3 | 3.2 % |
| 10 ng/mL | 146.9 | 3.3 | 2.3 % | 146.9 | 4.0 | 2.7 % |

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

| 5 ng/mL 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 |

Accuracy: 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:

Semi-Quantitative Accuracy Study:

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

The following table summarizes the result for the discordant samples:

| 5 ng/mL Cutoff | Norfentanyl LC/MS (ng/mL) | LC/MS | LZI EIA (ng/mL) | LZI EIA |
|----------------|---------------------------|-------|-----------------|---------|
| 38* | 1.5 | Neg | 6.1 | Pos |
| 44** | 3.0 | Neg | 6.0 | Pos |
| 46** | 3.3 | Neg | 9.6 | Pos |
| 47** | 3.5 | Neg | 14.0 | Pos |
| 48** | 3.8 | Neg | 18.5 | Pos |
| 50** | 4.2 | Neg | 9.6 | Pos |
| 52** | 4.6 | Neg | 15.7 | Pos |

Qualitative Accuracy Study:

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

The following table summarizes the result for the discordant samples:

| 5 ng/mL Cutoff | 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 |

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 | 100.0 % | 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, 1,3-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 % | Pos |
| 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, 1,3-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 Control | 6.25 ng/mL Control |
| (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 |
| Benzoyllecgonine | 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 |

Structurally Unrelated Compounds, continued:

| Compound | Spiked [] (ng/mL) | Spiked Norfentanyl Concentration | | |
|---|--------------------|----------------------------------|--------------------|--------------------|
| | | 0 ng/mL | 3.75 ng/mL Control | 6.25 ng/mL Control |
| 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-methylenedioxy-methamphetamine) | 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 false positive results.

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 | 40,000 | Pos | Pos | Pos |

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 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 |

Endogenous and Preservative Compound Interference Study, continued:

The following endogenous compounds which showed interference at $\pm 25\%$ of cutoff concentrations were then spiked into negative urine and at $\pm 50\%$ 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 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: 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: 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 and semi-quantitative modes. No interference was observed.

Open-Vial Reagent and Calibrator/Control Stability: Real-time data for open-vial reagent and calibrator/control stability studies at Cold Temperature (2-8°C) 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 2-8°C for maximum shelf life.

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

Symbols Used

| | | | |
|---|------------------------------------|---|--|
|  | Authorized Representative |  | Lot Number |
|  | Biological Risks |  | Manufacturer |
|  | CE Mark |  | R ₁ , Antibody/Substrate Reagent |
|  | Consult Instructions for Use |  | R ₂ , Enzyme-Drug Conjugate Reagent |
|  | Contents |  | Reference Number |
|  | Country of Origin |  | Safety Data Sheet |
|  | Date of Manufacture |  | Temperature Limits |
|  | Global Trade Item Number |  | Test Kit Number |
|  | In Vitro Diagnostic medical device |  | Use-by Date |

Additional Information

For more detailed information on 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 performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

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Shipping Damage

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

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

For instructions for use (including translations) please visit:

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