

# LZI Opiate 2000 Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only



REF 0330 (100/37.5 mL R<sub>1</sub>/R<sub>2</sub> Kit)  
0331 (1000/375 mL R<sub>1</sub>/R<sub>2</sub> Kit)



## Lin-Zhi International, Inc.

### Intended Use

The Lin-Zhi International, Inc. (LZI) Opiate 2000 Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of morphine in human urine at a cutoff value of 2000 ng/mL. 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 analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (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

Opiates are naturally occurring alkaloids derived from the opium poppy, *Papaver somniferum* (3). Common opiates include morphine, codeine and heroin, which is a semi-synthetic derivative of morphine. Morphine and codeine are potent analgesics. They are among the most effective and common medications for treatment of mild to severe pain. However, these prescription drugs are frequently abused for their central nervous system (CNS) effects. Heroin is the most commonly abused opiate (4). It may be snorted, smoked, or dissolved and injected subcutaneously or intravenously. Opiates are absorbed rapidly and primarily metabolized in liver (4-6). Heroin is converted quickly to 6-acetylmorphine or morphine, which is excreted in urine both unchanged and as glucuronide conjugates. Excretion takes place over 2 to 3 days. Codeine is excreted in urine as glucuronides, norcodeine, or as morphine. The presence of opiates in urine indicates the use of heroin, morphine, codeine, and/or other synthetic opiates structurally related to morphine, such as hydromorphone and hydrocodone.

### Assay Principle

The LZI Opiate 2000 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 (7). 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, opiate-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 binds to free drug; the unbound opiate-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 a 340 nm primary wavelength.

### Reagents Provided

**Antibody/Substrate Reagent (R<sub>1</sub>):** Contains mouse monoclonal anti-morphine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

**Enzyme-drug Conjugate Reagent (R<sub>2</sub>):** Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with morphine in buffer with sodium azide (0.09 %) as a preservative.

### Calibrators and Controls\*

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

| OPIATE 2000 Calibrators                               | REF  |
|---|------|
| Negative Calibrator                                   | 0001 |
| Low Calibrator: Contains 1000 ng/mL morphine          | 0332 |
| Cutoff Calibrator: Contains 2000 ng/mL morphine       | 0333 |
| Intermediate Calibrator: Contains 4000 ng/mL morphine | 0334 |
| High Calibrator: Contains 6000 ng/mL morphine         | 0335 |
| OPIATE 2000 Controls                                  | REF  |
| Level 1 Control: Contains 1500 ng/mL morphine         | 0337 |
| Level 2 Control: Contains 2500 ng/mL morphine         | 0338 |

### 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 (8).
- Do not use the reagents beyond their expiration dates.
- For USA: Caution: 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.

### Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (9). 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 seven days (10). For longer storage, keep sample frozen at -20°C and then thaw before use. Studies have shown opiate analytes in urine are stable at -20°C up to 12 months (11, 12). Samples should be at 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 the laboratory for testing.

Handle all urine specimens as if they are potentially infectious.

### Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength 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 Hitachi 717 and Beckman Coulter AU480. If other instruments are used, performance will need to be validated by the laboratory (13, 14).

### 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. Typical assay parameters used for the Hitachi 717 analyzer include a 8 µL sample, 200 µL of antibody reagent (R<sub>1</sub>), and 75 µL of enzyme conjugate reagent (R<sub>2</sub>) in 37°C incubation temperature, 30-35 reading frames, and a 340 nm primary wavelength. For qualitative analysis use the 2000 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 1500 ng/mL and 2500 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, guidelines and regulations.

### Results

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

**Qualitative:** The cutoff calibrator which contains 2000 ng/mL of morphine is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance ( $\Delta A/\text{min}$ ) equal to or greater than that obtained with the cutoff calibrator is considered a

preliminary positive. A sample with a change in absorbance ( $\Delta$ mA/min) 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 opiates in the sample may then be estimated from the calibration curve.

### Limitations

1. A preliminary positive result from the assay indicates only the presence of opiates. The test is not intended for quantifying this single analyte in samples.
2. A preliminary positive result does not necessarily indicate drug abuse.
3. A negative result does not necessarily mean a person did not take illegal drugs.
4. Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.
5. Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.
6. The test is designed for use with human urine only.
7. The test is not for therapeutic drug monitoring.

### Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer.

#### Precision:

Qualitative analysis: The three calibrators and two levels of controls were evaluated. Typical results (mA/min) are as follows:

| Concentration | Within Run (N=22) |     |       | Total Precision (N=88) |     |       |
|---------------|-------------------|-----|-------|------------------------|-----|-------|
|               | Mean              | SD  | % CV  | Mean                   | SD  | % CV  |
| 0 ng/mL       | 229.2             | 1.7 | 0.7 % | 229.2                  | 1.9 | 0.8 % |
| 500 ng/mL     | 271.2             | 1.7 | 0.6 % | 271.2                  | 2.2 | 0.8 % |
| 1000 ng/mL    | 335.9             | 1.5 | 0.4 % | 335.9                  | 2.7 | 0.8 % |
| 1500 ng/mL    | 384.7             | 1.7 | 0.4 % | 384.7                  | 2.8 | 0.7 % |
| 2000 ng/mL    | 414.5             | 2.7 | 0.7 % | 414.5                  | 3.5 | 0.9 % |
| 2500 ng/mL    | 441.9             | 1.9 | 0.4 % | 441.9                  | 3.1 | 0.7 % |
| 3000 ng/mL    | 461.9             | 3.4 | 0.7 % | 461.9                  | 4.2 | 0.9 % |
| 3500 ng/mL    | 478.6             | 3.0 | 0.6 % | 478.6                  | 3.5 | 0.7 % |
| 4000 ng/mL    | 489.4             | 2.2 | 0.5 % | 489.4                  | 2.9 | 0.6 % |

| 2000 ng/mL Cutoff |             | Within Run (N=22) |                  | Total Precision (N=88) |                   |
|-------------------|-------------|-------------------|------------------|------------------------|-------------------|
| Concentration     | % of Cutoff | # Samples         | EIA Result       | # Samples              | EIA Result        |
| 0 ng/mL           | 0 %         | 22                | 22 Neg           | 88                     | 88 Neg            |
| 500 ng/mL         | 25 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 1000 ng/mL        | 50 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 1500 ng/mL        | 75 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 2000 ng/mL        | 100 %       | 22                | 13 Neg/<br>9 Pos | 88                     | 55 Neg/<br>33 Pos |
| 2500 ng/mL        | 125 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 3000 ng/mL        | 150 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 3500 ng/mL        | 175 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 4000 ng/mL        | 200 %       | 22                | 22 Pos           | 88                     | 88 Pos            |

**Semi-quantitative analysis:** The concentrations of the cutoff level and the two levels of controls were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

| Concentration | Within Run (N=22) |      |        | Total Precision (N=88) |       |        |
|---------------|-------------------|------|--------|------------------------|-------|--------|
|               | Mean              | SD   | % CV   | Mean                   | SD    | % CV   |
| 0 ng/mL       | 64.9              | 21.4 | 36.6 % | 64.9                   | 25.7  | 39.6 % |
| 500 ng/mL     | 448.9             | 18.1 | 4.1 %  | 448.9                  | 22.1  | 4.9 %  |
| 1000 ng/mL    | 1014.4            | 24.2 | 2.4 %  | 1014.4                 | 24.8  | 2.4 %  |
| 1500 ng/mL    | 1559.8            | 20.1 | 1.3 %  | 1559.8                 | 22.6  | 1.4 %  |
| 2000 ng/mL    | 2018.4            | 32.2 | 1.6 %  | 2018.4                 | 37.6  | 1.9 %  |
| 2500 ng/mL    | 2492.3            | 46.4 | 1.9 %  | 2492.3                 | 47.0  | 1.9 %  |
| 3000 ng/mL    | 3065.5            | 87.9 | 2.9 %  | 3065.5                 | 90.1  | 2.9 %  |
| 3500 ng/mL    | 3608.7            | 76.9 | 2.2 %  | 3608.7                 | 110.4 | 3.1 %  |
| 4000 ng/mL    | 4013.2            | 84.0 | 2.1 %  | 4013.2                 | 96.9  | 2.4 %  |

| 2,000 ng/mL Cutoff |             | Within Run (N=22) |                  | Total Precision (N=88) |                   |
|--------------------|-------------|-------------------|------------------|------------------------|-------------------|
| Concentration      | % of Cutoff | # Samples         | EIA Result       | # Samples              | EIA Result        |
| 0 ng/mL            | 0 %         | 22                | 22 Neg           | 88                     | 88 Neg            |
| 500 ng/mL          | 25 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 1000 ng/mL         | 50 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 1500 ng/mL         | 75 %        | 22                | 22 Neg           | 88                     | 88 Neg            |
| 2000 ng/mL         | 100 %       | 22                | 4 Neg/<br>18 Pos | 88                     | 29 Neg/<br>59 Pos |
| 2500 ng/mL         | 125 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 3000 ng/mL         | 150 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 3500 ng/mL         | 175 %       | 22                | 22 Pos           | 88                     | 88 Pos            |
| 4000 ng/mL         | 200 %       | 22                | 22 Pos           | 88                     | 88 Pos            |

**Limit of Detection:** Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 200 ng/mL.

**Accuracy:** One-hundred-fifty (150) unaltered clinical urine specimens were tested with the LZI Opiate 2000 Enzyme Immunoassay and confirmed with GC/MS or LC/MS. Specimens having a morphine concentration greater than 2,000 ng/mL by GC/MS or LC/MS are defined as positive, and specimens with lower concentrations by GC/MS or LC/MS are defined as negative in the table below. Adjusted GC/MS or LC/MS values corrected for cross-reactivity, molecular weight, and excretion rates (15, 16). The correlation results are summarized as follows (near cutoff samples are defined as  $\pm$  50 % of the cutoff value):

#### Qualitative Accuracy Study:

| 2000 ng/mL Cutoff | Neg | < 50 % below the cutoff | Near Cutoff Neg | Near Cutoff Pos | > 50 % above the cutoff | % Agreement |
|-------------------|-----|-------------------------|-----------------|-----------------|-------------------------|-------------|
| Positive          | 0   | 0                       | 4*              | 19              | 33                      | 100.0 %     |
| Negative          | 20  | 43                      | 31              | 0               | 0                       | 95.8 %      |

The following table summarizes the result for the discordant samples:

| 2000 ng/mL Cutoff | Assay Result   |         | Sample Testing Method           |                  |
|-------------------|----------------|---------|---------------------------------|------------------|
|                   | GC/MS or LC/MS | LZI EIA | Adjusted GC/MS or LC/MS (ng/mL) | LZI EIA (mA/min) |
| Sample #88*       | -              | +       | 1708                            | 411.0            |
| Sample #90*       | -              | +       | 1726                            | 412.9            |
| Sample #94*       | -              | +       | 1853                            | 411.5            |
| Sample #96*       | -              | +       | 1869                            | 415.7            |

Discordant samples are based on a 2,000 ng/mL cutoff concentration with 408.8 mA/min absorbance values.

#### Semi-Quantitative Accuracy Study:

| 2000 ng/mL Cutoff | Neg | < 50 % below the cutoff | Near Cutoff Neg | Near Cutoff Pos | > 50 % above the cutoff | % Agreement |
|-------------------|-----|-------------------------|-----------------|-----------------|-------------------------|-------------|
| Positive          | 0   | 0                       | 8*              | 19              | 33                      | 100.0 %     |
| Negative          | 20  | 43                      | 27              | 0               | 0                       | 91.7 %      |

The following table summarizes the result for the discordant samples:

| 2000 ng/mL Cutoff | Assay Result   |         | Sample Testing Method           |                 |
|-------------------|----------------|---------|---------------------------------|-----------------|
|                   | GC/MS or LC/MS | LZI EIA | Adjusted GC/MS or LC/MS (ng/mL) | LZI EIA (ng/mL) |
| Sample #66*       | -              | +       | 1160                            | 2,020.4         |
| Sample #82*       | -              | +       | 1708                            | 2,115.8         |
| Sample #89*       | -              | +       | 1715                            | 2,073.5         |
| Sample #90*       | -              | +       | 1726                            | 2,067.1         |
| Sample #91*       | -              | +       | 1745                            | 2,062.0         |
| Sample #93*       | -              | +       | 1854                            | 2,062.1         |
| Sample #94*       | -              | +       | 1853                            | 2,180.1         |
| Sample #96*       | -              | +       | 1869                            | 2,116.3         |

**Analytical Recovery:** To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free urine pool spiked with morphine was serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follow:

$$y = 1.078x - 85.072, r^2 = 0.993$$

| Expected Value (ng/mL) | Observed Value (ng/mL) | % Recovery |
|------------------------|------------------------|------------|
| 6000                   | 6811.5                 | 113.5 %    |
| 5400                   | 5837.7                 | 108.1 %    |
| 4800                   | 4881.5                 | 101.7 %    |
| 4200                   | 4239.4                 | 100.9 %    |
| 3600                   | 3622.1                 | 100.6 %    |
| 3000                   | 3008.0                 | 100.3 %    |
| 2400                   | 2380.2                 | 99.2 %     |
| 1800                   | 1842.9                 | 102.4 %    |
| 1200                   | 1252.9                 | 104.4 %    |
| 600                    | 596.8                  | 99.5 %     |
| 200                    | 214.4                  | 107.2 %    |
| 0                      | 80.7                   | N/A        |

**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 table listed the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator or the

maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator.

#### Structurally Related Opiate Compounds:

| Compound                       | Target [ ] (ng/mL) | Observed Value (ng/mL) | % Cross-Reactivity |
|--------------------------------|--------------------|------------------------|--------------------|
| 6-Monoacetyl Morphine          | 2000               | 2234.7                 | 111.74 %           |
| Codeine                        | 1900               | 2164.4                 | 113.92 %           |
| Dextromethorphan               | 400,000            | 439.9                  | 0.110 %            |
| Dihydrocodeine                 | 7000               | 2226.4                 | 31.81 %            |
| Heroin                         | 2600               | 2079.1                 | 79.97 %            |
| Hydrocodone                    | 15,000             | 2345.6                 | 15.64 %            |
| Hydromorphone                  | 12,500             | 2354.0                 | 18.83 %            |
| Levorphanol                    | 56,000             | 2175.0                 | 3.88 %             |
| Morphine                       | 2000               | 2036.8                 | 101.84 %           |
| Morphine-3-Glucuronide         | 5000               | 2002.9                 | 40.06 %            |
| Morphine-6-Glucuronide         | 2500               | 2098.2                 | 83.93 %            |
| Nalbuphine                     | 30,000             | 64.4                   | 0.215 %            |
| Naloxone                       | 9000               | 66.5                   | 0.739 %            |
| Naltrexone                     | 1,200,000          | 477.3                  | 0.040 %            |
| Norcodeine                     | 305,000            | 2020.4                 | 0.66 %             |
| Normorphine                    | 30,000             | 239.1                  | 0.797 %            |
| Oxycodone                      | 600,000            | 2240.8                 | 0.37 %             |
| Oxymorphone                    | 1,020,000          | 2033.5                 | 0.20 %             |
| Thebaine                       | 15,000             | 2022.8                 | 13.49 %            |
| Codeine-6-β-Glucuronide        | 2000               | 2159.0                 | 107.95 %           |
| Norhydrocodone                 | 1,000,000          | 1497.2                 | 0.15 %             |
| Hydromorphone-3B-D-Glucuronide | 50,000             | 2068.7                 | 4.14 %             |

#### Structurally Unrelated Pharmacological Compounds:

| Compound             | Target [ ] (ng/mL) | Observed Value (ng/mL) | % Cross-Reactivity |
|----------------------|--------------------|------------------------|--------------------|
| Acetaminophen        | 3,000,000          | 0.0                    | 0.000 %            |
| Acetylsalicylic Acid | 2,000,000          | 0.0                    | 0.000 %            |
| Albuterol            | 70,000             | 0.0                    | 0.000 %            |
| Amitriptyline        | 25,000             | 0.0                    | 0.000 %            |
| Amobarbital          | 300,000            | 0.0                    | 0.000 %            |
| d-Amphetamine        | 3,000,000          | 121.8                  | 0.004 %            |
| Benzoylcegonine      | 3,000,000          | 0.0                    | 0.000 %            |
| Bupropion            | 3,000,000          | 75.8                   | 0.003 %            |
| Caffeine             | 1,500,000          | 0.0                    | 0.000 %            |
| Carbamazepine        | 3,000,000          | 77.0                   | 0.003 %            |
| Chlorpromazine       | 5000               | 0.0                    | 0.000 %            |
| Clomipramine         | 500,000            | 623.3                  | 0.125 %            |
| Desipramine          | 1000               | 61.9                   | 6.190 %            |
| Doxepine             | 50,000             | 108.7                  | 0.217 %            |
| Ecgonine             | 3,000,000          | 0.0                    | 0.000 %            |
| Ephedrine            | 3,000,000          | 211.8                  | 0.007 %            |
| Fentanyl             | 300                | 0.0                    | 0.000 %            |
| Fluoxetine           | 60,000             | 0.0                    | 0.000 %            |
| Fluphenazine         | 750,000            | 401.9                  | 0.054 %            |
| Ibuprofen            | 3,000,000          | 61.7                   | 0.002 %            |
| Imipramine           | 200,000            | 449.6                  | 0.225 %            |
| Lidocaine            | 60,000             | 99.6                   | 0.166 %            |
| Maprotiline          | 75,000             | 84.5                   | 0.113 %            |
| Meperidine           | 30,000             | 262.8                  | 0.876 %            |
| Methadone            | 400,000            | 529.2                  | 0.132 %            |
| Methapyrilene        | 600,000            | 590.1                  | 0.098 %            |
| Methaqualone         | 51,000             | 0.0                    | 0.000 %            |
| Metronidazole        | 700,000            | 0.0                    | 0.000 %            |
| Nicotine             | 10,000             | 25.7                   | 0.257 %            |
| Nortriptyline        | 360,000            | 84.8                   | 0.024 %            |
| Oxazepam             | 3,000,000          | 0.0                    | 0.000 %            |
| Phencyclidine        | 360,000            | 0.0                    | 0.000 %            |
| Phenobarbital        | 120,000            | 0.0                    | 0.000 %            |
| Propoxyphene         | 110,000            | 0.0                    | 0.000 %            |
| Ranitidine           | 318,000            | 208.1                  | 0.065 %            |
| Secobarbital         | 100,000            | 0.0                    | 0.000 %            |
| Talwin               | 100,000            | 0.0                    | 0.000 %            |
| Thioridazine         | 6000               | 0.0                    | 0.000 %            |
| Tramadol             | 330,000            | 550.8                  | 0.167 %            |
| Valproic Acid        | 2,000,000          | 64.2                   | 0.003 %            |

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

#### Endogenous Compound Interference Study:

| Interfering Substance | Spiked [ ] (mg/dL) | Spiked Morphine Concentration |            |            |
|-----------------------|--------------------|-------------------------------|------------|------------|
|                       |                    | 0 ng/mL                       | 1500 ng/mL | 2500 ng/mL |
| Acetone               | 1000               | 0.0                           | 1664.0     | 2714.9     |
| Ascorbic Acid         | 1500               | 0.0                           | 1697.1     | 2833.9     |
| Creatinine            | 500                | 0.0                           | 1750.7     | 2733.7     |
| Ethanol               | 1000               | 21.7                          | 1793.7     | 2507.5     |
| Galactose             | 10                 | 11.3                          | 1898.0     | 2558.6     |
| γ-Globulin            | 500                | 18.9                          | 1652.6     | 2744.5     |
| Glucose               | 3000               | 0.0                           | 1826.9     | 2683.5     |
| Hemoglobin            | 300                | 44.3                          | 1719.4     | 2770.3     |
| Human Serum Albumin   | 500                | 31.5                          | 1643.9     | 2728.5     |
| Oxalic Acid           | 100                | 12.3                          | 1635.0     | 2567.6     |
| Riboflavin            | 0.3                | 31.9                          | 1468.8     | 2407.9     |
| Sodium Chloride       | 6000               | 15.3                          | 1666.8     | 2632.5     |
| Urea                  | 6000               | 30.3                          | 1677.6     | 2564.3     |

#### pH Interference Study:

| pH    | Spiked Morphine Concentration |            |            |
|-------|-------------------------------|------------|------------|
|       | 0 ng/mL                       | 1500 ng/mL | 2500 ng/mL |
| pH 3  | 2.7                           | 1633.6     | 2691.8     |
| pH 4  | 21.9                          | 1690.0     | 2620.4     |
| pH 5  | 0.0                           | 1600.8     | 2647.0     |
| pH 6  | 0.0                           | 1688.7     | 2577.8     |
| pH 7  | 20.4                          | 1636.0     | 2546.2     |
| pH 8  | 6.0                           | 1612.2     | 2651.5     |
| pH 9  | 0.0                           | 1629.5     | 2606.9     |
| pH 10 | 0.0                           | 1644.6     | 2659.3     |
| pH 11 | 3.7                           | 1612.6     | 2592.6     |

**Specific Gravity:** Samples ranging in specific gravity from 1.000 to 1.025 were tested in qualitative mode against pooled processed urine samples at 0 ng/mL, 1500 ng/mL, or 2500 ng/mL (negative calibrator, negative control, and positive control for the 2000 ng/mL cutoff). No interference was observed.

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