LZI Methadone Metabolite (EDDP) Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0190b (100/37.5 mL R_1/R_2 Kit)
0191b (1000/375 mL R_1/R_2 Kit)

2°C 8°C

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

Intended Use

The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay is an *in vitro* diagnostic test intended for the qualitative and semi-quantitative determination of methadone metabolite in human urine at a cutoff value of 100 ng/mL and 300 ng/mL when calibrated against methadone metabolite. The assay is designed for screening with the Beckman Coulter AU480 clinical chemistry analyzer.

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 gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) or (2) permitting laboratories to establish quality control procedures.

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) is the preferred confirmatory method (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

Methadone is a synthetic diphenylheptanonylamine opioid that has similar analgesic activity and potency as morphine when administered parenterally. However, unlike morphine, it reliably retains its effectiveness when given orally, and tolerance and physical dependency develop slowly (3, 4).

Although methadone is prescribed to relieve chronic pain, its primary, medical application, however, is the detoxification and/or maintenance treatment of narcotic or heroin addiction (3, 5, 6). The abuse potential of methadone is comparable to that of morphine due to its similar pharmacological activity (3, 5, 7).

Methadone is readily absorbed from the gastrointestinal tract when ingested, and metabolized extensively in the liver. Initial N-demethylation results in normethadone metabolite, which rapidly undergoes cyclization followed by dehydration to form its major metabolite, 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine, commonly known as EDDP. Further N-demethylation yields a secondary metabolite, 2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline (EMDP). The metabolites are secreted in urine or bile along with unchanged drug (8).

Assay Principle

The LZI Methadone Metabolite Enzyme Immunoassay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between EDDP in the sample and EDDP labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (9). 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 EDDP in the sample, EDDP-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when EDDP is present in the sample, antibody binds to the free EDDP; the unbound G6PDH labeled with EDDP 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_1): Contains mouse monoclonal anti-EDDP 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 EDDP 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.*

METHADONE METABOLITE (EDDP) 100 ng/mL Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 50 ng/mL methadone metabolite	0202b
Cutoff Calibrator: Contains 100 ng/mL methadone metabolite	0203b
Intermediate Calibrator: Contains 250 ng/mL methadone metabolite	0204b
High Calibrator: Contains 500 ng/mL methadone metabolite	0205b

METHADONE METABOLITE (EDDP) 100 ng/mL Controls	REF
Level 1 Control: Contains 75 ng/mL methadone metabolite	0207b
Level 2 Control: Contains 125 ng/mL methadone metabolite	0208b

METHADONE METABOLITE (EDDP) 300 ng/mL Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 150 ng/mL methadone metabolite	0192b
Cutoff Calibrator: Contains 300 ng/mL methadone metabolite	0193b
Intermediate Calibrator: Contains 600 ng/mL methadone metabolite	0194b
High Calibrator: Contains 1000 ng/mL methadone metabolite	0195b

METHADONE METABOLITE (EDDP) 300 ng/mL Controls	REF
Level 1 Control: Contains 225 ng/mL methadone metabolite	0197b
Level 2 Control: Contains 375 ng/mL methadone metabolite	0198b

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 (10).*
- *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

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (11). Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days. For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown EDDP analytes in urine are stable at -20ºC up to six months (12). Samples should be at 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 forward both samples 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 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 AU480.

Assay Procedure

Please refer to the specific parameters used for each analyzer before performing the assay.

For 100 ng/mL Cutoff: Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 8 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 at 37ºC incubation temperature, 12-16 reading frame, FIXED method, and 340 nm primary wavelength.

For qualitative analysis use the 100 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. 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: 75 ng/mL and 125 ng/mL.

Assay Procedure, continued

For 300 ng/mL Cutoff: Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 4 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 at 37ºC incubation temperature, 12-16 reading frame, FIXED method, and 340 nm primary wavelength.

For qualitative analysis use the 300 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. 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: 225 ng/mL and 375 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 illegal drugs, and a negative test result does not necessarily mean a person did not take illegal drugs. 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 either 100 ng/mL or 300 ng/mL of methadone metabolite (EDDP), 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 methadone metabolite in the sample may then be estimated from the calibration curve. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of methadone metabolite. 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 Beckman Coulter AU480 automated chemistry analyzer.

Precision for 100 ng/mL Cutoff:
Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

100 ng/mL Cutoff Result:		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
25 ng/mL	25%	22	22 Neg	88	88 Neg
50 ng/mL	50%	22	22 Neg	88	88 Neg
75 ng/mL	75%	22	22 Neg	88	88 Neg
100 ng/mL	100%	22	11 Neg/ 11 Pos	88	48 Neg/ 40 Pos
125 ng/mL	125%	22	22 Pos	88	88 Pos
150 ng/mL	150%	22	22 Pos	88	88 Pos
175 ng/mL	175%	22	22 Pos	88	88 Pos
200 ng/mL	200%	22	22 Pos	88	88 Pos

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

100 ng/mL Cutoff Result:		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
25 ng/mL	25%	22	22 Neg	88	88 Neg
50 ng/mL	50%	22	22 Neg	88	88 Neg
75 ng/mL	75%	22	22 Neg	88	88 Neg
100 ng/mL	100%	22	13 Neg/ 9 Pos	88	54 Neg/ 34 Pos
125 ng/mL	125%	22	22 Pos	88	88 Pos
150 ng/mL	150%	22	22 Pos	88	88 Pos
175 ng/mL	175%	22	22 Pos	88	88 Pos
200 ng/mL	200%	22	22 Pos	88	88 Pos

Precision for 300 ng/mL Cutoff:
Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

300 ng/mL Cutoff Result:		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
75 ng/mL	25%	22	22 Neg	88	88 Neg
150 ng/mL	50%	22	22 Neg	88	88 Neg
225 ng/mL	75%	22	22 Neg	88	88 Neg
300 ng/mL	100%	22	6 Neg/ 16 Pos	88	36 Neg/ 52 Pos
375 ng/mL	125%	22	22 Pos	88	88 Pos
450 ng/mL	150%	22	22 Pos	88	88 Pos
525 ng/mL	175%	22	22 Pos	88	88 Pos
600 ng/mL	200%	22	22 Pos	88	88 Pos

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

300 ng/mL Cutoff Result:		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
75 ng/mL	25%	22	22 Neg	88	88 Neg
150 ng/mL	50%	22	22 Neg	88	88 Neg
225 ng/mL	75%	22	22 Neg	88	88 Neg
300 ng/mL	100%	22	7 Neg/ 15 Pos	88	33 Neg/ 55 Pos
375 ng/mL	125%	22	22 Pos	88	88 Pos
450 ng/mL	150%	22	22 Pos	88	88 Pos
525 ng/mL	175%	22	22 Pos	88	88 Pos
600 ng/mL	200%	22	22 Pos	88	88 Pos

Accuracy for 100 ng/mL Cutoff: Eighty-seven **(**87) unaltered clinical urine specimens were tested with LZI Methadone Metabolite (EDDP) Enzyme Immunoassay and confirmed with LC/MS. Specimens having a methadone metabolite concentration greater than 100 ng/mL by LC/MS are defined as positive, and specimens with concentrations lower than 100 ng/mL by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ±50% of the cutoff value):
Qualitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50% below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50% above the cutoff
Positive	0	0	0	2	40
Negative	23	11	9	2*	0

The following table summarizes the result for the two discrepant samples:

Sample #	Assay Result:		LC/MS (ng/mL)
	LC/MS	LZI EIA	
Sample #45*	+	-	103.1
Sample #46*	+	-	126.0

*Values are discrepant between the cutoff and 50% above the cutoff concentration (100 – 149.9 ng/mL)
Semi-Quantitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50% below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50% above the cutoff
Positive	0	0	0	2	40
Negative	23	11	9	2*	0

The following table summarizes the result for the two discrepant samples:

Sample #	Assay Result:		LC/MS (ng/mL)
	LC/MS	LZI EIA	
Sample #45*	+	-	103.1
Sample #46*	+	-	126.0

*Values are discrepant between the cutoff and 50% above the cutoff concentration (100 – 149.9 ng/mL)

Accuracy for 300 ng/mL Cutoff: Eighty-four (84) unaltered clinical urine specimens were tested with LZI Methadone Metabolite (EDDP) Enzyme Immunoassay and confirmed with LC/MS. Specimens having a methadone metabolite concentration greater than 300 ng/mL by LC/MS are defined as positive, and specimens with concentrations lower than 300 ng/mL by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ±50% of the cutoff value):

Qualitative Accuracy Study:

300 ng/mL Cutoff	Neg	<50% below the cutoff	Near Cutoff Neg	Near Cutoff Pos	>50% above the cutoff
Positive	0	0	0	4	38
Negative	21	15	6	0	0

Semi-Quantitative Accuracy Study:

300 ng/mL Cutoff	Neg	<50% below the cutoff	Near Cutoff Neg	Near Cutoff Pos	>50% above the cutoff
Positive	0	0	0	4	38
Negative	21	15	6	0	0

Analytical Recovery for 100 ng/mL Cutoff: To demonstrate linearity for purposes of sample dilution and quality control, a drug-free pool of urine was spiked with methadone metabolite to 500 ng/mL and was subsequently diluted. Each sample was run in ten replicates and the average was used determine percent recovery compared to the expected target value. Results are listed in the table below:

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100%	0	-3.1	N/A
98%	10	5.2	51.7%
90%	50	49.0	97.9%
80%	100	95.8	95.8%
75%	150	153.7	102.5%
60%	200	203.0	101.5%
50%	250	245.8	98.3%
40%	300	312.7	104.2%
30%	350	369.1	105.5%
20%	400	412.9	103.2%
10%	450	443.0	98.4%
0%	500	478.2	95.6%

Analytical Recovery for 300 ng/mL Cutoff: To demonstrate linearity for purposes of sample dilution and quality control, a drug-free pool of urine was spiked with methadone metabolite to 1000 ng/mL and was subsequently diluted. Each sample was run in ten replicates and the average was used determine percent recovery compared to the expected target value. Results are listed in the table below:

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100%	0	-6.4	N/A
98%	20	5.8	29.0%
90%	100	83.1	83.1%
80%	200	196.2	98.1%
75%	300	295.9	98.6%
60%	400	422.5	105.6%
50%	500	519.0	103.8%
40%	600	595.3	99.2%
30%	700	709.4	101.3%
20%	800	807.1	100.9%
10%	900	878.4	97.6%
0%	1000	940.9	94.1%

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator. The table listed below shows 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 did not exhibit interference.

Methadone Metabolite and Structurally Related Compounds for 100 ng/mL Cutoff:

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-Reactivity
EDDP	100	Pos	100%
EMDP	100000	Neg	<0.1%
Methadone	300000	Neg	<0.1%
LAAM HCl	500000	Neg	<0.1%
(±)-α-Methadol	300000	Neg	<0.1%
(-)-Isomethadone HCl	60000	Neg	<0.2%
(-)-α-Noracetylmethadol (Nor-LAAM) HCl	300000	Neg	<0.1%

Structurally Unrelated Pharmacological Compounds for 100 ng/mL Cutoff:

The table below lists the maximal concentration of the compound tested without interference.

Interfering Substances	Spiked [ ] (ng/mL)
Acetaminophen	100000
6-Acetylmorphine	10000
Acetylsalicylic Acid	100000
Alimemazine	1000
Amitriptyline	100000
Amlodipine	100000
Amoxicillin	100000
d-Amphetamine	100000
Atorvastatin	20000
Benzoylecgonine	100000
Buprenorphine	15000
Bupropion	100000
Caffeine	100000
Carbamazepine	100000
Cetirizine	100000
Chlorpheniramine	100000
Chlorpromazine	50000
Clomipramine	100000
Codeine	100000
Cyamemazine	12000
Desipramine	100000
Diphenhydramine	100000
Doxylamine	100000
Duloxetine	20000
Fentanyl	10000
Fluoxetine	100000
Fluphenazine	100000
Gabapentin	100000
Hydrocodone	100000
Hydromorphone	100000
Ibuprofen	100000
Imipramine	100000
Levomepromazine	40000
Lisinopril	100000
Losartan	10000
Loratadine	100000
MDA (3,4-Methylene-Dioxyamphetamine)	100000
MDEA	100000
MDMA (3,4-Methylene-Dioxymethylamphetamine)	100000
Meperidine	100000
Metformin	100000
Methylphenidate	100000
Metoprolol	100000
d-Methamphetamine	100000
Methapyrilene	10000
Methaqualone	100000
Metronidazole	100000
Morphine	100000
Nicotine	100000
Nortriptyline	100000
Omeprazole	100000
Oxazepam	100000
Oxycodone	100000
Oxymorphone	100000
Phencyclidine	10000
Phenobarbital	100000
Promethazine	5000
(1S,2S)-(+) Pseudoephedrine	100000
Quetiapine	100000
Ranitidine	100000
Salbutamol (Albuterol)	100000
Sertraline	5000
THC-COOH (11-Nor-Delta-9-THC-9-Carboxylic Acid)	1000
Thioridazine	20000
l-Thyroxine	10000
Tramadol	100000
Verapamil	100000
Zolpidem	10000

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

Methadone Metabolite and Structurally Related Compounds for 300 ng/mL Cutoff:

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross-Reactivity
EDDP	300	Pos	100%
EMDP	100000	Neg	<0.1%
Methadone	500000	Neg	<0.1%
LAAM HCl	500000	Neg	<0.1%
(±)-α-Methadol	300000	Neg	<0.1%
(-)-Isomethadone HCl	200000	Neg	<0.1%
(-)-α-Noracetylmethadol (Nor-LAAM) HCl	300000	Neg	<0.1%

Structurally Unrelated Pharmacological Compounds for 300 ng/mL Cutoff:

The table listed below shows the maximal concentration of the compound tested without interference.

Interfering Substances	Spiked [ ] (ng/mL)
Acetaminophen	100000
6-Acetylmorphine	10000
Acetylsalicylic Acid	100000
Alimemazine	4000
Amitriptyline	100000
Amlodipine	100000
Amoxicillin	100000
d-Amphetamine	100000
Atorvastatin	20000
Benzoylecgonine	100000
Buprenorphine	15000
Bupropion	100000
Caffeine	100000
Carbamazepine	100000
Cetirizine	100000
Chlorpheniramine	100000
Chlorpromazine	100000
Clomipramine	100000
Codeine	100000
Cyamemazine	25000
Desipramine	100000
Diphenhydramine	100000
Doxylamine	100000
Duloxetine	60000
Fentanyl	10000
Fluoxetine	100000
Fluphenazine	100000
Gabapentin	100000
Hydrocodone	100000
Hydromorphone	100000
Ibuprofen	100000
Imipramine	100000
Levomepromazine	100000
Lisinopril	100000
Losartan	10000
Loratadine	100000
MDA (3,4-Methylene-Dioxyamphetamine)	100000
MDEA	100000
MDMA (3,4-Methylene-Dioxymethylamphetamine)	100000
Meperidine	100000
Metformin	100000
Methylphenidate	100000
Metoprolol	100000
d-Methamphetamine	100000
Methapyrilene	40000
Methaqualone	100000
Metronidazole	100000
Morphine	100000
Nicotine	100000
Nortriptyline	100000
Omeprazole	100000
Oxazepam	100000
Oxycodone	100000
Oxymorphone	100000
Phencyclidine	20000
Phenobarbital	100000
Promethazine	15000
(1S,2S)-(+)Pseudoephedrine	100000
Quetiapine	100000
Ranitidine	100000
Salbutamol (Albuterol)	100000
Sertraline	15000

Structurally Unrelated Pharmacological Compounds for 300 ng/mL Cutoff, continued:

Interfering Substances	Spiked [ ] (ng/mL)
THC-COOH (11-Nor-Delta-9-THC-9-Carboxylic Acid)	1000
Thioridazine	90000
l-Thyroxine	10000
Tramadol	100000
Verapamil	100000
Zolpidem	10000

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

Interference: Endogenous Substances for 100 ng/mL Cutoff

The following potentially interfering compounds were spiked into a pool of processed drug-free urine to the desired concentrations and then EDDP was spiked to a final concentration of 0 ng/mL or the negative control concentration of 75 ng/mL, or the positive control concentration of 125 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding preliminary positive/negative results against the cutoff value of 100 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference.

Interfering Substances	Spiked [ ] (mg/dL)
Acetone	1000
Ascorbic Acid	1500
Creatinine	500
Ethanol	1000
Galactose	10
γ-Globulin	500
Glucose	3000
Hemoglobin	300
Human Serum Albumin	500
Oxalic Acid	100
Riboflavin	7.5
Urea	6000
Sodium Chloride	4000
pH 3	N/A
pH 4	N/A
pH 5	N/A
pH 6	N/A
pH 7	N/A
pH 8	N/A
pH 9	N/A
pH 10	N/A
pH 11	N/A

Interference: Endogenous Substances for 300 ng/mL Cutoff

The following potentially interfering compounds were spiked into a pool of processed drug-free urine to the desired concentrations and then EDDP was spiked to a final concentration of 0 ng/mL or the negative control concentration of 225 ng/mL, or the positive control concentration of 375 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding preliminary positive/negative results against the cutoff value of 300 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference.

Interfering Substances	Spiked [ ] (mg/dL)
Acetone	1000
Ascorbic Acid	1500
Creatinine	500
Ethanol	1000
Galactose	10
γ-Globulin	500
Glucose	3000
Hemoglobin	300
Human Serum Albumin	500
Oxalic Acid	100
Riboflavin	7.5
Urea	6000
Sodium Chloride	6000
pH 3	N/A
pH 4	N/A
pH 5	N/A
pH 6	N/A

Interference: Endogenous Substances for 300 ng/mL Cutoff, continued:

Interfering Substances	Spiked [ ] (mg/dL)
pH 7	N/A
pH 8	N/A
pH 9	N/A
pH 10	N/A
pH 11	N/A

Specific Gravity for 100 ng/mL Cutoff: Samples ranging in specific gravity from 1.004 to 1.025 were spiked with methadone metabolite to a final concentration of 0 ng/mL, the negative control concentration of 75 ng/mL, or the positive control concentration of 125 ng/mL. No interference was observed.

Specific Gravity for 300 ng/mL Cutoff: Samples ranging in specific gravity from 1.004 to 1.025 were spiked with methadone metabolite to a final concentration of 0 ng/mL, the negative control concentration of 225 ng/mL, or the positive control concentration of 375 ng/mL. No interference was observed.

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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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