

LZI Methadone II Enzyme Immunoassay



0630 (100/37.5 mL R₁/R₂ Kit)
0631 (1000/375 mL R₁/R₂ Kit)



IVD For In Vitro Diagnostic Use Only



Lin-Zhi International, Inc.

Intended Use

The LZI Methadone II Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of methadone in human urine at a cutoff value of 300 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

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) 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

Methadone is a synthetic diphenylheptanonylamine opioid that has similar analgesic activity and potency to 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 application is the detoxification and/or treatment of narcotic or heroin addiction (3-6). The abuse potential of methadone is comparable to that of morphine due to its similar pharmacological activity (3, 5, 7).

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

Assay Principle

The LZI Methadone II Enzyme Immunoassay 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 (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 drug in the sample, methadone-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 methadone-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 (R₁): Contains mouse monoclonal anti-methadone 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 methadone 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.

METHADONE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 150 ng/mL methadone	0112
Cutoff Calibrator: Contains 300 ng/mL methadone	0113
Intermediate Calibrator: Contains 600 ng/mL methadone	0114
High Calibrator: Contains 1000 ng/mL methadone	0115
METHADONE Controls	REF
Level 1 Control: Contains 225 ng/mL methadone	0117
Level 2 Control: Contains 375 ng/mL methadone	0118

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.
- 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 (11). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2-8°C for up to seven days (12). For longer storage, keep sample frozen at -20°C and then thaw before use. Studies have shown methadone analytes in urine are stable at -20°C up to 384 days (13). 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 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 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 Beckman Coulter AU680. If other instruments are used, performance will need to be validated by the laboratory (14, 15).

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 Beckman Coulter AU680 analyzer include a 12 µL sample, 120 µL of antibody reagent (R₁), and 45 µL of enzyme conjugate reagent (R₂) in 37°C incubation temperature, 13-17 reading frame, FIXED method, and a 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 if there is 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.

Qualitative: The cutoff calibrator which contains 300 ng/mL of methadone 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 methadone in the sample may then be estimated from the calibration curve.

Limitations

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

Typical Performance Characteristics

The results shown below were obtained with the Beckman Coulter AU680 clinical chemistry analyzer.

Precision:

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

300 ng/mL Cutoff		Within Run (N=22)		Total Precision (N=88)	
Methadone Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
75 ng/mL	-25.0 %	22	22 Neg	88	88 Neg
150 ng/mL	50.0 %	22	22 Neg	88	88 Neg
225 ng/mL	75.0 %	22	22 Neg	88	88 Neg
300 ng/mL	100.0 %	22	17 Neg/ 5 Pos	88	59 Neg/ 29 Pos
375 ng/mL	125.0 %	22	22 Pos	88	88 Pos
450 ng/mL	150.0 %	22	22 Pos	88	88 Pos
525 ng/mL	175.0 %	22	22 Pos	88	88 Pos
600 ng/mL	200.0 %	22	22 Pos	88	88 Pos

Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results were measured in ng/mL. Positive/Negative results are as follows:

300 ng/mL Cutoff		Within Run (N=22)		Total Precision (N=88)	
Methadone Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
75 ng/mL	25.0 %	22	22 Neg	88	88 Neg
150 ng/mL	50.0 %	22	22 Neg	88	88 Neg
225 ng/mL	75.0 %	22	22 Neg	88	88 Neg
300 ng/mL	100.0 %	22	18 Neg/ 4 Pos	88	66 Neg/ 22 Pos
375 ng/mL	125.0 %	22	22 Pos	88	88 Pos
450 ng/mL	150.0 %	22	22 Pos	88	88 Pos
525 ng/mL	175.0 %	22	22 Pos	88	88 Pos
600 ng/mL	200.0 %	22	22 Pos	88	88 Pos

Accuracy: Ninety-four (94) clinical urine specimens were tested with the LZI Methadone II Enzyme Immunoassay and confirmed with LC/MS. Specimens having a methadone concentration greater than or equal to 300 ng/mL by LC/MS were defined as positive, and specimens with total concentrations below 300 ng/mL by LC/MS were defined as negative in the table below. Near cutoff samples are defined as \pm 50 % of the cutoff value. The correlation results are summarized as follows:

Semi-Quantitative Accuracy Study:

300 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
Positive	0	0	1*	4	41	97.8 %
Negative	20	23	4	1**	0	97.9 %

The following table summarizes the result for the discordant samples:

300 ng/mL Cutoff	MTD LC/MS (ng/mL)	LC/MS Result	LZI MTD II EIA Result
Sample #48*	274	-	+
Sample #50**	317	+	-

Qualitative Accuracy Study:

300 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
Positive	0	0	1*	4	41	97.8 %
Negative	20	23	4	1**	0	97.9 %

The following table summarizes the result for the discordant samples:

300 ng/mL Cutoff	MTD LC/MS (ng/mL)	LC/MS Result	LZI MTD II EIA (mAU)	LZI EIA	Qualitative Cutoff Rate (mAU)
Sample #48*	274	-	176.5	+	133.5
Sample #50**	317	+	95.4	-	165.0

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section) of the entire assay range, a drugfree urine pool spiked with methadone at 1000 ng/mL was serially diluted. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value. Samples from the linear range of the assay (100 ng/mL to 1000 ng/mL) were tested with recovery ranging from 93.7% to 104.9%.

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
1000	950.5	95.0 %
900	848.6	94.3 %
800	767.9	96.0 %
700	668.0	95.4 %
600	572.2	95.4 %
500	463.0	92.6 %
400	393.4	98.4 %
300	287.8	95.9 %
200	187.4	93.7 %
100	104.9	104.9 %
0	-7.1	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 below 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).

Structurally Related Methadone Compounds:

Cross-reactant	Concentration Tested (ng/mL)	% Cross-reactivity
Methadone	300	100.00 %
EDDP	100,000	<0.30 %
EMDP	100,000	<0.30 %
(-)- α -Noracetylmethadol (Nor-LAAM) HCl	75,000	0.40 %
LAAM HCl	25,000	1.20 %
(\pm)- α -Methadol	26,500	1.13 %
(-)-Isomethadone HCl	35,000	0.86 %

Structurally Unrelated Pharmacological Compounds:

Cross-reactant	Spiked [] (ng/mL)	Spiked Methadone Concentration		
		0 ng/mL	225 ng/mL Control	375 ng/mL Control
Acetaminophen	100,000	ND	Neg	Pos
6-Acetylmorphine	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
d-Amphetamine	100,000	ND	Neg	Pos
Atorvastatin	100,000	ND	Neg	Pos
Benzoylcegonine	100,000	ND	Neg	Pos
Buprenorphine	100,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
Desipramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Duloxetine	50,000	ND	Neg	Pos
Fentanyl	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
Losartan	100,000	ND	Neg	Pos
Loratadine	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylenedioxymethamphetamine)	100,000	ND	Neg	Pos
Meperidine	100,000	ND	Neg	Pos
Metformin	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
d-Methamphetamine	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
Phenobarbital	100,000	ND	Neg	Pos
(1S,2S)-(+)-Pseudoephedrine	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
L-Thyroxine	100,000	ND	Neg	Pos
Tramadol	100,000	ND	Neg	Pos
Zolpidem	100,000	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.

Endogenous and Preservative Compound Interference Study:

The following endogenous compounds were spiked into pooled negative human urine and the two levels of controls (225 ng/mL and 375 ng/mL) for the assay. These spiked solutions were then evaluated in semi-quantitative and qualitative modes.

Interference was observed with Boric Acid. No other major interference was found as all other spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 300 ng/mL. Results are summarized in the following table:

Endogenous Substance	Concentration Tested (mg/dL)	Spiked Methadone Concentration		
		0 ng/mL	225 ng/mL Control	375 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	Pos
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	0.3	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	1000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos

The following endogenous compounds which showed interference at ±25 % of the cutoff concentrations were then spiked into negative urine at ±50 % of the cutoff concentrations (150 ng/mL and 450 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	Concentration Tested (mg/dL)	Spiked Methadone Concentration		
		0 ng/mL	150 ng/mL	450 ng/mL
Boric Acid	1000	Neg	Neg	Neg
Potassium Chloride	6000	Neg	Neg	Pos
















pH Interference Study: Negative urine and urine spiked with analyte to the two levels of controls (225 ng/mL and 375 ng/mL) were adjusted to the following pH levels and tested by the assay. These spiked solutions were then evaluated in semi-quantitative and qualitative modes.

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 300 ng/mL. Results are summarized in the following table:

pH	Spiked Methadone Concentration		
	0 ng/mL	225 ng/mL Control	375 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 methadone concentration of either 225 ng/mL or 375 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in qualitative mode. No interference was observed.

Symbols Used

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

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

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