LZI Methadone Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0110 (100/37.5 mL R_1/R_2 Kit)
0111 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Methadone 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 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 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 (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 (R_1): 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 (R_2): 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.*
- Rx ONLY *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 2-8ºC 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 2-8ºC for up to seven days (12). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown methadone analytes in urine are stable at -20ºC up to 384 days (13). 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 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 Synchron® CX4CE and the Synermed IR500 clinical analyzers.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. 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 (ΔmA/min) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmA/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 methadone 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 methadone. 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 obtained with the Synchron CX4CE clinical chemistry analyzer.

Precision:

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

Concentration	Within Run (N=21)			Run-to-Run* (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	209.4	1.0	0.5 %	209.5	1.1	0.5 %
225 ng/mL	272.8	1.1	0.4 %	271.4	2.0	0.7 %
300 ng/mL	293.4	0.7	0.3 %	292.3	1.9	0.7 %
375 ng/mL	308.2	0.9	0.3 %	307.2	2.6	0.8 %
1000 ng/mL	344.9	1.1	0.3 %	344.1	2.0	0.6 %

**Run-to-Run testing completed over 3 weeks*

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=21)			Run-to-Run* (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
225 ng/mL	229.2	2.5	1.1 %	228.0	7.8	3.4 %
300 ng/mL	308.5	3.5	1.1 %	305.1	10.2	3.4 %
375 ng/mL	391.4	5.4	1.4 %	379.6	13.2	3.5 %

**Run-to-Run testing completed over 3 weeks*

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 15 ng/mL.

Accuracy: One hundred and sixty (160) clinical urine specimens were tested with current EIA, 49 samples were positive, and 111 samples were negative. All positive samples were confirmed with GC/MS results.

Cutoff Value (300 ng/mL)	GC/MS	LZI MTD EIA	% Agreement with Predicate
# Positive Samples	49	49	100 %
# Negative Samples	111	111	100 %
Total # of Samples	160	160	N/A

In addition to the above study, 19 diluted clinical samples with a methadone concentration ranging from 205 ng/mL to 434 ng/mL (determined by GC/MS) were evaluated with the LZI Methadone EIA. The eight samples with methadone GC/MS values greater than the cutoff (ranging from 305 to 434 ng/mL) tested positive by EIA. Among the 11 samples with methadone GC/MS below the cutoff (ranging from 205 to 288 ng/mL), three samples were found negative while eight samples tested positive by the EIA. The GC/MS concentrations of methadone in these eight samples range from 214 ng/mL to 288 ng/mL. Each of these samples also contained a substantial amount of the primary methadone metabolite EDDP (ranging from 247 ng/mL to 825 ng/mL).

Analytical Recovery: Analytical recovery was evaluated by spiking known concentrations of methadone to negative urine samples.
In qualitative analysis, the assay correctly identified spiked samples containing more than 300 ng/mL of methadone (n=25, spiked levels equal to or higher than Level 2 Control, 375 ng/mL) as positive, and those containing less than 300 ng/mL of methadone (n=25, spiked levels equal to or less than Level 1 Control, 225 ng/mL) as negative.
For semi-quantitative analysis, the average recovery for samples spiked with 30 to 900 ng/mL of methadone (five samples at each level) is summarized in the following table:

Analytical Recovery, continued:

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
30	29.5	98.5 %
60	64.2	106.9 %
120	122.9	102.5 %
180	176.3	97.9 %
225	217.3	96.6 %
375	351.5	93.7 %
400	460.5	92.1 %
600	531.3	88.5 %
750	638.6	85.1 %
900	700.8	77.9 %

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).

Structurally Related Methadone Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Methadone	0.30	Positive
LAAM-HCl	10	Positive
(-) α-Methadol-HCl	8	Positive
nor-LAAM-HCl	10	Negative
EDDP-HI	100	Negative
EMDP-HCl	100	Negative

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Acetaminophen	1000	Negative
Acetylsalicylic Acid	1000	Negative
Amitriptyline	1000	Negative
Amobarbital	1000	Negative
Amphetamine	1000	Negative
Benzoylecgonine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine	1000	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Ephedrine	1000	Negative
Ibuprofen	2000	Negative
Imipramine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	1000	Negative
Morphine	1000	Negative
Nortriptyline	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Promethazine	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Valproic Acid	1000	Negative

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

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