LZI Oxycodone III Enzyme Immunoassay

For 300 ng/mL Cutoff

REF 0610 (100/37.5 mL R_1/R_2 Kit)
0611 (1000/375 mL R_1/R_2 Kit) 2°C 8°C

IVD For In Vitro Diagnostic Use Only

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oxycodone III Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of oxycodone in human urine at a cutoff value of 300 ng/mL when calibrated against oxycodone. 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 verification by a confirmatory method such as 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 chemical confirmatory method (e.g., gas or liquid chromatography and mass spectrometry) must be used 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

Oxycodone is a semi-synthetic narcotic analgesic prescribed for pain management in patients with moderate to severe pain. The drug is approximately equipotent with morphine, but has a higher oral/parenteral dose (3). Similar to morphine, oxycodone can produce drug tolerance and therefore has the potential of being abused. Oxycodone is metabolized by N- and O-demethylation into oxymorphone and noroxycodone. The oxymorphone metabolite is a potent narcotic analgesic and the noroxycodone is relatively inactive. Between 33-61 % of a single dose of oxycodone is excreted in the 24-hour urine as free drug (13-19 %) and conjugated oxycodone (7-29 %), conjugated oxymorphone (13-14 %) and an unknown amount of noroxycodone (3).

Assay Principle

The LZI Oxycodone III Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. 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. The drug-labeled G6PDH conjugate is traceable to a commercially available oxycodone standard and referred to as oxycodone-labeled G6PDH conjugate. 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, oxycodone-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when free drug is present in the sample, antibody would bind to free drug; the unbound oxycodone-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 (R_1): Contains mouse monoclonal anti-oxycodone 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 oxycodone 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.*

OXYCODONE 300 ng/mL Cutoff Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 150 ng/mL oxycodone	0622
Cutoff Calibrator: Contains 300 ng/mL oxycodone	0623
Intermediate Calibrator: Contains 500 ng/mL oxycodone	0624
High Calibrator: Contains 800 ng/mL oxycodone	0625

OXYCODONE 300 ng/mL Cutoff Controls	REF
Level 1 Control: Contains 225 ng/mL oxycodone	0627
Level 2 Control: Contains 375 ng/mL oxycodone	0628

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 (5).*
- *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. Use of plastics such as polyethylene is recommended (6). 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 oxycodone analytes in urine are stable at -20ºC for up to six months (7). 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 Beckman Coulter AU480. If other instruments are used, performance will need to be validated by the laboratory.

Assay Procedure

Analyzers with 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 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 in 37ºC incubation temperature, 14-18 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 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 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 300 ng/mL of oxycodone, is used as a reference for distinguishing a preliminary positive from negative samples. A sample with a change in absorbance (ΔmAU) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmAU) 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 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 oxycodone and oxymorphone 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 oxycodone. The test is not intended for quantifying these single analytes 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 must be confirmed by other affirmative, analytical 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 clinical chemistry analyzer.

Precision:

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		Within Run (N=22)		Total Precision (N=88)	
Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0 %	22	22 Negative	88	88 Negative
75 ng/mL	25 %	22	22 Negative	88	88 Negative
150 ng/mL	50 %	22	22 Negative	88	88 Negative
225 ng/mL	75 %	22	22 Negative	88	88 Negative
300 ng/mL	100 %	22	6 Neg / 16 Pos	88	27 Neg / 61 Pos
375 ng/mL	125 %	22	22 Positive	88	88 Positive
450 ng/mL	150 %	22	22 Positive	88	88 Positive
525 ng/mL	175 %	22	22 Positive	88	88 Positive
600 ng/mL	200 %	22	22 Positive	88	88 Positive

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

300 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 Negative	88	88 Negative
75 ng/mL	25 %	22	22 Negative	88	88 Negative
150 ng/mL	50 %	22	22 Negative	88	88 Negative
225 ng/mL	75 %	22	22 Negative	88	88 Negative
300 ng/mL	100 %	22	10 Neg / 12 Pos	88	40 Neg / 48 Pos
375 ng/mL	125 %	22	22 Positive	88	88 Positive
450 ng/mL	150 %	22	22 Positive	88	88 Positive
525 ng/mL	175 %	22	22 Positive	88	88 Positive
600 ng/mL	200 %	22	22 Positive	88	88 Positive

Accuracy: Ninety (90) unaltered clinical urine specimens were tested with the LZI Oxycodone III Enzyme Immunoassay and confirmed with LC/MS. Specimens having an oxycodone and oxymorphone concentration greater than or equal to 300 ng/mL by LC/MS are defined as positive, and specimens with an oxycodone and oxymorphone concentration below 300 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:

300 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	2*	11	33	97.8 %
Negative	20	6	17	1**	0	95.6 %

The following table summarizes the result for the semi-quantitative discordant samples:

Sample #	Oxycodone LC/MS (ng/mL)	Oxymorphone LC/MS (ng/mL)	Total Oxycodone + Oxymorphone LC/MS (ng/mL)	Pos/ Neg Result	AU480 EIA Semi-Quantitative Result (ng/mL)	Pos/ Neg Result
42*	200.2	49.5	249.7	-	438.7	+
43*	53.0	203.5	256.5	-	363.2	+
55**	46.7	367.3	414.0	+	264.5	-

Qualitative Accuracy Study:

300 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	%Agree-ment
Positive	0	0	2*	10	33	95.6 %
Negative	20	6	17	2**	0	95.6 %

The following table summarizes the results for the qualitative discordant samples:

Sample #	Oxycodone LC/MS (ng/mL)	Oxymorphone LC/MS (ng/mL)	Total Oxycodone + Oxymorphone LC/MS (ng/mL)	Pos/ Neg Result	AU480 EIA Qualitative Result (mAU)	Pos/ Neg Result	Qualitative Cutoff Rate (mAU)
42*	200.2	49.5	249.7	-	568.7	+	514.7
43*	53.0	203.5	256.5	-	540.4	+	514.2
50**	177.2	212.1	389.3	+	508.5	-	514.2
55**	46.7	367.3	414.0	+	490.0	-	514.2

Analytical Recovery: To demonstrate recovery for purposes of sample dilution and quality control of the entire assay range, a drug free–urine pool spiked with oxycodone at 800 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.

Target Concentration (ng/mL)	Determined Concentration Range (ng/mL)	Determined Concentration Average (ng/mL)	Average % Recovery
800	829.6 – 859.3	843.5	105.4 %
720	769.7 – 794.1	784.2	108.9 %
640	689.6 – 731.2	712.8	111.4 %
560	603.8 – 640.0	624.3	111.5 %
480	497.8 – 525.2	514.2	107.1 %
400	427.2 – 451.5	436.8	109.2 %
320	327.6 – 359.1	345.0	107.8 %
240	242.9 – 260.8	250.8	104.5 %
160	168.3 – 183.4	173.9	108.7 %
80	82.9 – 95.0	89.0	111.2 %
0	-5.2 – 5.9	0.3	N/A

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into a drug free–urine pool to various concentrations and evaluated with the assay's calibration curve in both qualitative and semi-quantitative modes.
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 (100,000 ng/mL) with results below the cutoff value were listed as Not Detected (ND). Compounds tested below the high concentration (100,000 ng/mL) that gave a result below the cutoff value were given a "< %" value.

Oxycodone and Major Metabolites:

Compound	Test Concentration (ng/mL)	% Cross-reactivity
Oxycodone	300	100.00 %
Oxymorphone	300	100.00 %
Noroxycodone	75,000	0.40 %
Noroxymorphone	100,000	ND

Structurally Related Compounds:

Compound	Test Concentration (ng/mL)	% Cross-Reactivity
6-Acetylmorphine	100,000	ND
Buprenorphine	100,000	ND
Codeine	100,000	ND
Codeine-6β-D-Glucuronide	100,000	ND
Dextromethorphan	100,000	ND
Dihydrocodeine	100,000	ND
Hydrocodone	75,000	0.40 %
Hydromorphone	75,000	0.40 %

Structurally Related Compounds, continued:

Compound	Test Concentration (ng/mL)	% Cross-Reactivity
Levorphanol	100,000	ND
Morphine	100,000	ND
Morphine-3β-D-Glucuronide	100,000	ND
Morphine-6β-D-Glucuronide	100,000	ND
Naloxone	100,000	ND
Naloxone-3β-D-Glucuronide	100,000	ND
Norbuprenorphine	100,000	ND
Norcodeine	100,000	ND
Norhydrocodone	100,000	ND
Oxymorphone-3β-D-Glucuronide	700	42.86 %

Structurally Unrelated Compounds:

Compound	Test Concentration (ng/mL)	Spiked Oxycodone Concentration		
		0 ng/mL	225 ng/mL Control	375 ng/mL Control
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
d-Amphetamine	100,000	ND	Neg	Pos
Atorvastatin	20,000	< 1.50 %	Neg	Pos
Benzoylecgonine	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
Desipramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Duloxetine	100,000	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
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos
Losartan	10,000	< 3.00 %	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
Methadone	100,000	ND	Neg	Pos
d-Methamphetamine	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
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)	1000	< 30.00 %	Neg	Pos
l-Thyroxine	10,000	< 3.00 %	Neg	Pos
Tramadol	100,000	ND	Neg	Pos
Zolpidem	10,000	< 3.00 %	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 Preservatives Compound Interference Study:
Various potentially interfering endogenous and preservative substances were tested for interference with the assay. Test compounds were split into three portions each and either left un-spiked or spiked to an oxycodone concentration of either 225 or 375 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes:

Endogenous or Preservative Substance	Concentration of Compound (mg/dL)	Spiked Oxycodone 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 ($CaCl_2$)	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	Pos
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
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate	300	Neg	Neg	Pos

The following endogenous compounds which showed interference at ±25 % of the cutoff concentration were then spiked into negative urine and at ±50 % of the cutoff concentration (150 ng/mL and 450 ng/mL) for the assay. Interference was not observed with Boric Acid at 1 % w/v at ±50 % of the cutoff concentration. Results are summarized in the following table:

Endogenous or Preservative Substance	Concentration of Compound (mg/dL)	Spiked Oxycodone Concentration		
		0 ng/mL	150 ng/mL	450 ng/mL
Boric Acid	1000	Neg	Neg	Pos

pH Interference Study: pH 3 to pH 11 was tested for interference with the assay. Each pH level was split into three portions each and either left un-spiked or spiked to an oxycodone concentration of either 225 or 375 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No pH interference was observed.

pH	Spiked Oxycodone 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 1.000 to 1.030 were split into three portions each and either left un-spiked or spiked to an oxycodone concentration of either 225 or 375 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No interference was observed.

Specific Gravity	Spiked Oxycodone Concentration		
	0 ng/mL	225 ng/mL Control	375 ng/mL Control
1.000	Neg	Neg	Pos
1.003	Neg	Neg	Pos
1.005	Neg	Neg	Pos
1.008	Neg	Neg	Pos
1.010	Neg	Neg	Pos
1.012	Neg	Neg	Pos
1.015	Neg	Neg	Pos
1.020	Neg	Neg	Pos
1.025	Neg	Neg	Pos
1.030	Neg	Neg	Pos

Symbols Used

EC REP	Authorized Representative		Manufacturer
	Biological Risks	REAGENT 1	R_1, Antibody/ Substrate Reagent
CE	CE Mark	REAGENT 2	R_2, Enzyme-Drug Conjugate Reagent
	Consult Instructions for Use	REF	Reference Number
CONTENTS	Contents	SDS	Safety Data Sheet
GTIN	Global Trade Item Number	8°C 2°C	Temperature Limits
IVD	*In Vitro* Diagnostic medical device	T.K.	Test Kit Number
LOT	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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