LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0370 (100/37.5 mL R_1/R_2 Kit)
0371 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of carisoprodol metabolite (meprobamate) in human urine at a cutoff value of 100 ng/mL when calibrated against meprobamate. 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, 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

Carisoprodol is a carbamate derivative that acts as a skeletal muscle relaxant and sedative. It is often prescribed as the free drug in combination with acetaminophen, aspirin, caffeine, or codeine for oral administration. Therapeutic use of carisoprodol produces adverse effects such as drowsiness, dizziness, and ataxia. Isolated case reports suggest that carisoprodol may have the potential for abuse (1-3). In 2007, the European Medicines Agency (EMEA) recommended a suspension for marketing carisoprodol products due to the risk of abuse or addiction.

Carisoprodol is known to be metabolized to meprobamate and hydroxymeprobamate (4). Although carisoprodol has significant pharmacological activity, its main benefit in therapy is likely due mostly to the meprobamate metabolite, as meprobamate remains in the system significantly longer and reaches a higher peak plasma concentration than the parent drug a few hours following administration (5). Meprobamate is also a drug with high abuse and addictive potential (6, 7).

Less than 1 % of a single 350 mg oral dose is excreted unchanged in the 24-hour urine, with meprobamate accounting for 4.7 % of the dose (8). Meprobamate and the hydroxylated metabolites may be partially excreted as conjugates (9). Depending on the frequency and dosage of carisporodol or meprobamate intake, meprobamate levels above the cutoff of this assay (100 ng/mL) may be present in urine for up to several days (10-23).

Assay Principle

The LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between meprobamate in the sample and the enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled with meprobamate for a fixed amount of antibody in the reagent (24). Enzyme activity decreases upon binding to the antibody, and the meprobamate concentration in the sample is measured in terms of enzyme activity. In the absence of meprobamate in the sample, meprobamate-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when meprobamate is present in the sample, antibody would bind to free meprobamate; the unbound meprobamate-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 a mouse monoclonal anti-meprobamate 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 meprobamate 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.*

MEPROBAMATE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 50 ng/mL meprobamate	0372
Cutoff Calibrator: Contains 100 ng/mL meprobamate	0373
Intermediate Calibrator: Contains 200 ng/mL meprobamate	0374
High Calibrator: Contains 400 ng/mL meprobamate	0375

MEPROBAMATE Controls	REF
Level 1 Control: Contains 75 ng/mL meprobamate	0377
Level 2 Control: Contains 125 ng/mL meprobamate	0378

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 (25).*
- *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 (26). Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to one week (27). For longer storage, keep sample frozen at -20ºC and then thaw before use (28). Internal sample stability studies have shown meprobamate samples in urine are stable at -20ºC for up to 17 months. Samples should be equilibrated to room temperature (18-25ºC) for testing.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to a 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® AU400e.

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU400e analyzer include a 12 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, 14-18 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.

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 100 ng/mL of meprobamate, is used as a reference for distinguishing 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 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, 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 meprobamate 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 this assay indicates only the presence of meprobamate and does not necessarily correlate with the extent of physiological and psychological effects (e.g., intoxication).*
3. *A negative result does not necessarily mean a person did not abuse 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 (28-30).*
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. *This test should not be used to monitor therapeutic drug concentrations or to inform dosing adjustment decisions.*

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU400e automated chemistry analyzer.

Precision:

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:

100 ng/mL Cutoff		Within Run (N=22)		Run-to-Run (N=88)	
Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	-100.0 %	22	22 Neg	88	88 Neg
25 ng/mL	-75.0 %	22	22 Neg	88	88 Neg
50 ng/mL	-50.0 %	22	22 Neg	88	88 Neg
75 ng/mL	-25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	0.0 %	22	14 Neg/ 8 Pos	88	60 Neg/ 28 Pos
125 ng/mL	+25.0 %	22	22 Pos	88	88 Pos
150 ng/mL	+50.0 %	22	22 Pos	88	88 Pos
175 ng/mL	+75.0 %	22	22 Pos	88	88 Pos
200 ng/mL	+100.0 %	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		Within Run (N=22)		Run-to-Run (N=88)	
Concentration	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	-100.0 %	22	22 Neg	88	88 Neg
25 ng/mL	-75.0 %	22	22 Neg	88	88 Neg
50 ng/mL	-50.0 %	22	22 Neg	88	88 Neg
75 ng/mL	-25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	0.0 %	22	9 Neg/ 13 Pos	88	40 Neg/ 48 Pos
125 ng/mL	+25.0 %	22	22 Pos	88	88 Pos
150 ng/mL	+50.0 %	22	22 Pos	88	88 Pos
175 ng/mL	+75.0 %	22	22 Pos	88	88 Pos
200 ng/mL	+100.0 %	22	22 Pos	88	88 Pos

Accuracy: One hundred twenty-seven (127) unaltered clinical urine specimens were tested with the LZI Carisoprodol Metabolite (Meprobamate) Enzyme Immunoassay and confirmed with GC/MS or LC/MS. Specimens having a meprobamate concentration greater than 100 ng/mL by GC/MS or LC/MS are defined as positive, and specimens with meprobamate concentration below 100 ng/mL by GC/MS or 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:

100 ng/mL Cutoff	Neg	< 50 % below the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agree-ment
Positive	0	0	1*	11	56	98.5 %
Negative	22	21	15	1**	0	98.3 %

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

Sample #	Meprobamate LC/MS (ng/mL)	Pos/Neg Result	AU400e EIA Semi-Quantitative Pos/Neg Result
59*	98	-	+
60**	103	+	-

Qualitative Accuracy Study:

100 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	56	98.5 %
Negative	22	21	14	1**	0	96.6 %

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

Sample#	Meprobamate GC/MS or LC/MS (ng/mL)	Pos/Neg Result	AU400e EIA Qualitative Result (mAU)	Pos/Neg Result	Qualitative Cutoff Rate (mAU)
58*	92	-	150.8	+	150.6
59*	98	-	129.3	+	123.0
60**	103	+	152.0	-	154.6

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section) of the entire assay range, a drug-free urine pool spiked with meprobamate at 400 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.

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
400	412.5	103.1 %
360	386.6	107.4 %
320	348.9	109.0 %
280	305.7	109.2 %
240	251.6	104.8 %
200	195.3	97.7 %
160	163.7	102.3 %
120	123.3	102.8 %
80	81.8	102.2 %
40	39.9	99.7 %
10	6.7	66.6 %

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 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).

Structurally Related Compounds:

Compound	Concentration (ng/mL)	% Cross-Reactivity
Carisoprodol	110	90.9 %
Darunavir	200,000	<0.1 %
Efavirenz	200,000	<0.1 %
Felbamate	400	25.0 %
Hydroxymeprobamate	65,000	0.2 %
Meprobamate	100	100.0 %
Meprobamate-N-Glucuronide	20,000	0.5 %
Methocarbamol	200,000	<0.1 %
Mitocycin C	200,000	<0.1 %
Neostigmine Bromide	200,000	<0.1 %
Retigabine	200,000	<0.1 %
Ritonavir	100,000	<0.1 %
Rivastigmine Tartrate	200,000	<0.1 %
Zafirlukast	200,000	<0.1 %

Structurally Unrelated Compounds:

Compound	Spiked [ ] (ng/mL)	Spiked Meprobamate Concentration		
		0 ng/mL (ng/mL)	75 ng/mL Control (ng/mL)	125 ng/mL Control (ng/mL)
Acetaminophen	100,000	Neg	Neg	Pos
6-Acetylmorphine	10,000	Neg	Neg	Pos
Acetylsalicylic Acid	100,000	Neg	Neg	Pos
Albuterol (Salbutamol)	100,000	Neg	Neg	Pos
Amitriptyline	100,000	Neg	Neg	Pos
d-Amphetamine	100,000	Neg	Neg	Pos
Benzoylecgonine	100,000	Neg	Neg	Pos
Buprenorphine	15,000	Neg	Neg	Pos
Bupropion	100,000	Neg	Neg	Pos
Caffeine	100,000	Neg	Neg	Pos
Carbamazepine	100,000	Neg	Neg	Pos
Cetirizine	20,000	Neg	Neg	Pos
Chlorpheniramine	100,000	Neg	Neg	Pos
Chlorpromazine	100,000	Neg	Neg	Pos
Clomipramine	100,000	Neg	Neg	Pos
Codeine	100,000	Neg	Neg	Pos
Cyclobenzaprine	100,000	Neg	Neg	Pos
Desipramine	100,000	Neg	Neg	Pos
Diphenhydramine	100,000	Neg	Neg	Pos
Ephedrine	100,000	Neg	Neg	Pos
Fentanyl	10,000	Neg	Neg	Pos
Fluoxetine	100,000	Neg	Neg	Pos
Fluphenazine	100,000	Neg	Neg	Pos
Hydrocodone	100,000	Neg	Neg	Pos
Hydromorphone	100,000	Neg	Neg	Pos
Ibuprofen	100,000	Neg	Neg	Pos
Imipramine	100,000	Neg	Neg	Pos
Lidocaine	100,000	Neg	Neg	Pos
Loratadine	100,000	Neg	Neg	Pos
Maprotiline	30,000	Neg	Neg	Pos
MDA (3,4-Methylenedioxyamphetamine)	100,000	Neg	Neg	Pos
MDEA	100,000	Neg	Neg	Pos
MDMA (3,4-Methylenedioxymethamphetamine)	100,000	Neg	Neg	Pos
Meperidine	100,000	Neg	Neg	Pos
Methadone	100,000	Neg	Neg	Pos
d-Methamphetamine	100,000	Neg	Neg	Pos
Methapyrilene	100,000	Neg	Neg	Pos
Methaqualone	100,000	Neg	Neg	Pos
Metronidazole	100,000	Neg	Neg	Pos
Morphine	100,000	Neg	Neg	Pos
Nicotine	100,000	Neg	Neg	Pos
Nortriptyline	100,000	Neg	Neg	Pos
Oxazepam	100,000	Neg	Neg	Pos
Oxycodone	100,000	Neg	Neg	Pos
Oxymorphone	100,000	Neg	Neg	Pos
Phencyclidine	10,000	Neg	Neg	Pos
Pentazocine	20,000	Neg	Neg	Pos
Phenobarbital	100,000	Neg	Neg	Pos
d-Propoxyphene	100,000	Neg	Neg	Pos
Propranaolol	100,000	Neg	Neg	Pos
Ranitidine	100,000	Neg	Neg	Pos
Sertraline	100,000	Neg	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-Carboxylic Acid)	1000	Neg	Neg	Pos
Thioridazine	100,000	Neg	Neg	Pos
Tramadol	100,000	Neg	Neg	Pos
Valproic Acid	100,000	Neg	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 unspiked or further spiked to a final meprobamate concentration of either 75 ng/mL or 125 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. Only the preservative Boric Acid (final concentration 1 %) was found to cause interference with the assay.

Endogenous or Preservative Substance	Spiked [ ] (mg/dL)	Spiked Meprobamate Concentration		
		0 ng/mL (ng/mL)	75 ng/mL Control (ng/mL)	125 ng/mL Control (ng/mL)
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Pos
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride ($CaCl_2$) Dihydrate (Saturated Solution)	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
Human Serum Albumin	500	Neg	Neg	Pos
β-hydoxybutyric Acid Sodium Salt	100	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassuium Chloride	6000	Neg	Neg	Pos
Riboflavin	7.5	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	6000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Neg	Pos
Sodium Phosphate Dibasic Salt	300	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid Monosodium Salt	10	Neg	Neg	Pos

pH Interference Study: The pH range from pH 3 to pH 11 was tested for interference with the assay. Each pH level was split into three portions each and either left unspiked or further spiked to a final meprobamate concentration of either 75 ng/mL or 125 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 Meprobamate Concentration		
	0 ng/mL (ng/mL)	75 ng/mL Control (ng/mL)	125 ng/mL Control (ng/mL)
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 unspiked or further spiked to a final meprobamate concentration of either 75 ng/mL or 125 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No interference was observed.

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

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