# LZI Ketamine Enzyme Immunoassay

**For 100 ng/mL Cutoff**

REF 0640 (100/37.5 mL $R_1/R_2$ Kit)
0641 (1000/375 mL $R_1/R_2$ Kit)

2-8ºC

IVD For In Vitro Diagnostic Use Only

***For Forensic Use Only***

***Lin-Zhi International, Inc.***

## Intended Use

The LZI Ketamine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of norketamine in human urine at the cutoff value of 100 ng/mL when calibrated against norketamine. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. This is a Non-FDA Approved assay for Forensic Use Only and as such should not be repackaged for in vitro diagnostic use.
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 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 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 a preliminary positive.**

## Summary and Explanation of Test

Ketamine (2-[2-chlorophenyl]-2-[methylamino]-cyclohexanone) is a pharmaceutical derived from phencyclidine (PCP) and cyclohexamine. Mechanistically, it acts as a non-competitive N-methyl-D-aspartate (NMDA)-receptor antagonist. The NMDA-receptor is involved in sensory input at the spinal, thalamic, limbic and cortical levels (3, 4).
Ketamine has been shown to have a number of beneficial pharmacological properties. It is primarily considered an anaesthetic with a good safety profile. (5) Its major drawback, limiting its clinical use, is the occurrence of emergence reactions or dissociative effects (e.g., hallucinations, vivid dreams, floating sensations and delirium.) (3, 6). Recently, extensive research has been carried out on the antidepressant properties of ketamine (7-9).
The frequent use of ketamine can lead to addiction and dependence (10). Ketamine posseses narcotic effects similar to phencyclidine (PCP) and hallucinogenic effects similar to lysergic acid diethylamide (LSD) (11, 12). The recreational use of ketamine as a rave, party, and nightclub drug has increased over time, thus increasing public concerns about the potential hazards of this drug (13-15).
Ketamine undergoes rapid N-demethylation by liver microsomal cytochrome P450 enzymes CYP 3A4, CYP 2B6, and CYP 2C9 to form its primary metabolite, norketamine, which is pharmacologically active, and an inactive metabolite, 6-hydroxynorketamine (16, 17). A small percentage of unchanged ketamine (2.3%), norketamine (1.6%), and dehydronorketamine (16.2%) are eliminated in urine, whereas 80% is present as the glucuronide conjugates of hydroxylated metabolites of ketamine (18-21). While dehydronorketamine is present at higher levels and for a longer period of time than ketamine and norketamine in urine, dehydronorketamine has a lower stability, potentially limiting its utility in the detection of ketamine abuse (22).

## Assay Principle

The LZI Ketamine 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 (23). The drug-labeled G6PDH conjugate is traceable to a commercially available ketamine standard and referred to as ketamine-labeled G6PDH conjugate. Enzyme activity decreases upon binding to the antibody, and the norketamine concentration in the sample is measured in terms of enzyme activity. In the absence of ketamine and/or norketamine in the sample, ketamine-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when ketamine and/or norketamine is present in the sample, antibody would bind to free ketamine and/or norketamine; the unbound ketamine-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-ketamine 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 ketamine in buffer with sodium azide (0.09 %) as a preservative.

## Calibrators and Controls*

**Calibrators and Controls are sold separately or as a semi-quantitative set and contain negative human urine with sodium azide as a preservative.*

| Norketamine Calibrators | REF |
|---|---|
| Negative Calibrator | 0001 |
| Low Calibrator: Contains 25 ng/mL norketamine | 0642 |
| Cutoff #1 Calibrator: Contains 50 ng/mL norketamine | 0643 |
| Cutoff #2 Calibrator: Contains 100 ng/mL norketamine | 0644 |
| Intermediate Calibrator: Contains 250 ng/mL norketamine | 0645 |
| High Calibrator: Contains 500 ng/mL norketamine | 0646 |

| Norketamine (100) Controls | REF |
|---|---|
| Level 1 Control: Contains 75 ng/mL norketamine | 0657 |
| Level 2 Control: Contains 125 ng/mL norketamine | 0658 |

## *Precautions and Warning*

- *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 (24).*
- *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.

## Specimen Collection and Handling

Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for seven days. For longer storage, keep sample frozen at -20ºC and then thaw before use (22). 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 AU480 automated clinical analyzer.

## Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis use the 100 ng/mL as the cutoff calibrator.
For semi-quantitative analysis, use all six calibrators including the universal negative calibrator. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are available for monitoring of each cutoff level. Use the 75 ng/mL and 125 ng/mL controls for the 100 ng/mL cutoff level.

## 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 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 norketamine, 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 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 six calibrators. The concentration of norketamine in the sample may then be estimated from the calibration curve.

## Limitations

1. *Phencyclidine at 100,000 ng/mL may cause false positive results.*
2. *Boric Acid at 1% w/v may cause false negative results.*
3. *A preliminary positive result from this assay indicates only the presence of norketamine. The test is not intended for quantifying this single analyte in samples.*
4. *A preliminary positive result does not necessarily indicate drug abuse.*
5. *A negative result does not necessarily mean a person did not take illegal drugs.*
6. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferants) may influence the urine test result.*
7. *Preliminary positive results must be confirmed by other affirmative, analytical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
8. *The test is designed for use with human urine only.*
9. *This test should not be used for therapeutic drug monitoring.*

## Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU480 automated chemistry analyzer.

**Precision:**

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 | | Within Run (N = 22) | | Run-to-Run (N = 88) | |
|---|---|---|---|---|---|
| **Norketamine 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 | 5 Neg/ 17 Pos | 88 | 28 Neg/ 60 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 | | Within Run (N = 22) | | Run-to-Run (N = 88) | |
|---|---|---|---|---|---|
| **Norketamine 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 | 3 Neg/ 19 Pos | 88 | 24 Neg/ 64 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 |

**Accuracy:** One hundred eleven (111) unaltered clinical urine specimens and pooled urine samples spiked with norketamine were tested with the LZI Ketamine Enzyme Immunoassay and confirmed with LC/MS. Specimens with a combined norketamine and ketamine concentration greater than or equal to 100 ng/mL by LC/MS are defined as positive, and specimens with a combined norketamine and ketamine concentration below 100 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:

| 100 ng/mL Cutoff | Neg | < 50 % of the cutoff | Near Cutoff Neg | Near Cutoff Pos | High Pos | % Agree-ment |
|---|---|---|---|---|---|---|
| Positive | 0 | 3* | 3** | 4 | 47 | 92.7 % |
| Negative | 20 | 21 | 9 | 4*** | 0 | 89.3 % |

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

| Sample # | NKET LC/MS (ng/mL) | KET LC/MS (ng/mL) | Total NKET + KET LC/MS (ng/mL) | Pos/ Neg Result | AU480 EIA Semi-Quantitative Result (ng/mL) | Pos/ Neg Result |
|---|---|---|---|---|---|---|
| 24* | 17.0 | 0.0 | 17.0 | - | 212.0 | + |
| 26* | 19.6 | 0.0 | 19.6 | - | 117.4 | + |
| 31* | 14.3 | 12.8 | 27.1 | - | 122.2 | + |
| 45** | 40.1 | 13.9 | 54.0 | - | 228.1 | + |
| 46** | 28.6 | 29.4 | 58.0 | - | 233.1 | + |
| 56** | 81.2 | 13.8 | 95.0 | - | 605.5 | + |
| 57*** | 104.0 | 0.0 | 104.0 | + | 81.7 | - |
| 58*** | 104.0 | 0.0 | 104.0 | + | 86.2 | - |
| 59*** | 110.0 | 0.0 | 110.0 | + | 86.4 | - |
| 60*** | 111.0 | 0.0 | 111.0 | + | 86.9 | - |

** Discordant between negative and <50 % cutoff concentration (0.1 – 49.9 ng/mL)*
*** Discordant between <50 % cutoff and cutoff concentration (50 – 99.9 ng/mL)*
**** Discordant between cutoff and >50 % cutoff concentration (100 – 149.9 ng/mL)*

Qualitative Accuracy Study:

| 100 ng/mL Cutoff | Neg | < 50 % of the cutoff | Near Cutoff Neg | Near Cutoff Pos | High Pos | % Agree-ment |
|---|---|---|---|---|---|---|
| Positive | 0 | 3* | 3** | 4 | 47 | 92.7 % |
| Negative | 20 | 21 | 9 | 4*** | 0 | 89.3 % |

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

| Sample # | NKET LC/MS (ng/mL) | KET LC/MS (ng/mL) | Total NKET + KET LC/MS (ng/mL) | Pos/ Neg Result | AU480 EIA Qualitative Result (ng/mL) | Pos/ Neg Result |
|---|---|---|---|---|---|---|
| 24* | 17.0 | 0.0 | 17.0 | - | 263.0 | + |
| 26* | 19.6 | 0.0 | 19.6 | - | 273.5 | + |
| 31* | 14.3 | 12.8 | 27.1 | - | 154.0 | + |
| 45** | 40.1 | 13.9 | 54.0 | - | 284.9 | + |
| 46** | 28.6 | 29.4 | 58.0 | - | 292.2 | + |
| 56** | 81.2 | 13.8 | 95.0 | - | 469.9 | + |
| 57*** | 104.0 | 0.0 | 104.0 | + | 96.2 | - |
| 58*** | 104.0 | 0.0 | 104.0 | + | 99.9 | - |
| 59*** | 110.0 | 0.0 | 110.0 | + | 101.1 | - |
| 60*** | 111.0 | 0.0 | 111.0 | + | 100.6 | - |

Calibration Cutoff Average = 123.3 mAU
** Discordant between negative and <50 % cutoff concentration (0.1 – 49.9 ng/mL)*
*** Discordant between <50 % cutoff and cutoff concentration (50 – 99.9 ng/mL)*
**** Discordant between cutoff and >50 % cutoff concentration (100 – 149.9 ng/mL)*

**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 norketamine at 500 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 |
|---|---|---|---|
| 500 | 497.6 - 511.8 | 503.2 | 100.6% |
| 450 | 460.5 - 482.3 | 470.5 | 104.6% |
| 400 | 427.1 - 440 | 431.8 | 107.9% |
| 350 | 374.7 - 399.1 | 385.1 | 110.0% |
| 300 | 315.9 - 333.6 | 325.5 | 108.5% |
| 250 | 241.5 - 249.1 | 246.4 | 98.6% |
| 200 | 202.2 - 211.1 | 206.8 | 103.4% |
| 150 | 150.3 - 161.1 | 153.1 | 102.1% |
| 100 | 94.3 - 99.4 | 96.8 | 96.8% |
| 50 | 47.7 - 52.1 | 49.5 | 99.0% |
| 7.5 | 6.3 - 12.4 | 8.0 | 106.4% |
| 0 | -1.4 - 4.2 | 1.0 | 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.

**Ketamine and Metabolites:**

| Cross-reactant | Concentration (ng/mL) | % Cross-reactivity |
|---|---|---|
| Norketamine | 100 | 100.00% |
| Ketamine | 100 | 100.00% |
| Dehydronorketamine | 4,000 | 2.50% |
| Deschloroketamine | 4,000 | 2.50% |
| Methoxetamine | 100,000 | 0.10% |
| Hydronorketamine | 100,000 | 0.10% |

**Structurally Unrelated Compounds:**

| Cross-reactant | Spiked [ ] (ng/mL) | Spiked Norketamine Concentration | | |
|---|---|---|---|---|
| | | 0 ng/mL | 75 ng/mL | 125 ng/mL |
| Acetaminophen | 100,000 | 0.10% | Neg | Pos |
| 6-Acetylmorphine (powder) | 100,000 | 0.10% | Neg | Pos |
| Acetylsalicylic Acid | 100,000 | 0.10% | Neg | Pos |
| Amitriptyline | 50,000 | 0.20% | Neg | Pos |
| Amlodipine Besylate | 100,000 | 0.10% | Neg | Pos |
| Amoxicillin | 100,000 | 0.10% | Neg | Pos |
| d-Amphetamine | 100,000 | 0.10% | Neg | Pos |
| Atorvastatin | 100,000 | 0.10% | Neg | Pos |
| Benzoylecgonine | 100,000 | 0.10% | Neg | Pos |
| Buprenorphine | 50,000 | 0.20% | Neg | Pos |
| Bupropion | 100,000 | 0.10% | Neg | Pos |
| Caffeine | 100,000 | 0.10% | Neg | Pos |
| Carbamazepine | 10,000 | 1.00% | Neg | Pos |
| Carbamazepine-10,11-epoxide | 10,000 | 1.00% | Neg | Pos |
| Cetirizine | 100,000 | 0.10% | Neg | Pos |
| Chlorpheniramine | 100,000 | 0.10% | Neg | Pos |
| Chlorpromazine | 10,000 | 1.00% | Neg | Pos |
| Clomipramine | 100,000 | 0.10% | Neg | Pos |
| Codeine | 100,000 | 0.10% | Neg | Pos |
| Desipramine | 100,000 | 0.10% | Neg | Pos |
| Diphenhydramine | 100,000 | 0.10% | Neg | Pos |
| Desipramine | 100,000 | 0.10% | Neg | Pos |
| (±)-10,11-Dihydro-10-Hydroxycarbamazepine | 10,000 | 1.00% | Neg | Pos |
| Diphenhydramine | 100,000 | 0.10% | Neg | Pos |
| Duloxetine | 100,000 | 0.10% | Neg | Pos |
| Fentanyl (citrate) | 10,000 | 0.20% | Neg | Pos |
| Fluoxetine | 100,000 | 0.10% | Neg | Pos |
| Fluphenazine | 100,000 | 0.10% | Neg | Pos |
| Gabapentin | 100,000 | 0.10% | Neg | Pos |
| Hydrocodone | 100,000 | 0.10% | Neg | Pos |
| Hydromorphone | 100,000 | 0.10% | Neg | Pos |
| Ibuprofen | 100,000 | 0.20% | Neg | Pos |
| Imipramine | 60,000 | 0.10% | Neg | Pos |
| Lisinopril | 100,000 | 0.10% | Neg | Pos |
| Losartan | 100,000 | 1.00% | Neg | Pos |
| Loratidine | 100,000 | 0.10% | Neg | Pos |
| MDA (3,4-methylenedioxyamphetamine) | 100,000 | 0.10% | Neg | Pos |
| MDEA (Methyl diethanolamine) | 100,000 | 1.00% | Neg | Pos |
| MDMA (3,4-methylenedioxy-methamphetamine) | 100,000 | 0.10% | Neg | Pos |
| Meperidine | 100,000 | 0.10% | Neg | Pos |
| Metformin | 100,000 | 0.10% | Neg | Pos |
| Metoprolol | 100,000 | 0.10% | Neg | Pos |
| Methadone | 100,000 | 0.10% | Neg | Pos |
| *d*-Methamphetamine | 100,000 | 1.00% | Neg | Pos |
| Morphine | 100,000 | 0.10% | Neg | Pos |
| Nalmefene | 100,000 | 0.10% | Neg | Pos |
| Negative Urine | N/A | ND | Neg | Pos |
| Nicotine | 100,000 | 0.10% | Neg | Pos |
| Norfentanyl | 10,000 | 1.00% | Neg | Pos |
| Nortriptyline | 100,000 | 0.10% | Neg | Pos |
| Omeprazole | 100,000 | 0.10% | Neg | Pos |
| Oxazepam | 100,000 | 0.10% | Neg | Pos |
| Oxycodone | 100,000 | 0.10% | Neg | Pos |
| Oxymorphone | 100,000 | 0.10% | Neg | Pos |
| Phencyclidine | 100,000 | 0.10% | Pos | Pos |
| Phenobarbital | 100,000 | 0.10% | Neg | Pos |

**Structurally Unrelated Compounds, continued:**

| Cross-reactant | Spiked [ ] (ng/mL) | Spiked Norketamine Concentration | | |
|---|---|---|---|---|
| | | 0 ng/mL | 75 ng/mL | 125 ng/mL |
| Promethazine | 15,000 | 0.67% | Neg | Pos |
| (1S,2S)-(+)Pseudoephedrine | 100,000 | 0.10% | Neg | Pos |
| Quetiapine | 50,000 | 0.20% | Neg | Pos |
| Ranitidine | 100,000 | 0.10% | Neg | Pos |
| Salbutamol (Albuterol) | 100,000 | 0.10% | Neg | Pos |
| Sertraline | 100,000 | 0.10% | Neg | Pos |
| THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid) | 100,000 | 0.10% | Neg | Pos |
| L-Thyroxine | 100,000 | 0.10% | Neg | Pos |
| Tramadol | 100,000 | 0.10% | Neg | Pos |
| Zolpidem | 10,000 | 1.00% | Neg | Pos |

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

The following compounds which showed interference at ±25 % of cutoff concentrations were then spiked into negative urine and at ±50 % of cutoff concentrations (50 ng/mL and 150 ng/mL) for the assay. Results are summarized in the following table:

| Cross-reactant | Spiked [ ] (ng/mL) | Spiked Norketamine Concentration | | |
|---|---|---|---|---|
| | | 0 ng/mL | 50 ng/mL | 150 ng/mL |
| Phencyclidine | 100,000 | 0.05% | Pos | Pos |

**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 a norketamine concentration of either 75 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 (1 % w/v) was found to cause interference with the assay.

| Endogenous or Preservative Substance | Spiked [ ] (mg/dL) | Spiked Norketamine Concentration | | |
|---|---|---|---|---|
| | | 0 ng/mL | 75 ng/mL | 125 ng/mL |
| Acetone | 1000 | Neg | Neg | Pos |
| Ascorbic Acid | 1500 | Neg | Neg | Pos |
| Bilirubin | 2 | Neg | Neg | Pos |
| Biotin | 0.5 | 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 |
| Negative Urine pool | N/A | Neg | Neg | Pos |
| Oxalic Acid | 100 | Neg | Neg | Pos |
| Potassium Chloride | 3000 | 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 | 3000 | Neg | Neg | Pos |
| Sodium Fluoride | 1000 | Neg | Neg | Pos |
| Sodium Phosphate | 300 | Neg | Neg | Pos |
| UbCB | N/A | Neg | Neg | Pos |
| Acetone | 1000 | Neg | Neg | Pos |

The following compound which showed interference at ±25 % of cutoff concentrations were then spiked into negative urine and at ±50 % of cutoff concentrations (50 ng/mL and 150 ng/mL) for the assay. Interference was still observed with Boric Acid. Results are summarized in the following table:

| Endogenous or Preservative Substance | Spiked [ ] (mg/dL) | Spiked Norketamine Concentration | | |
|---|---|---|---|---|
| | | 0 ng/mL | 50 ng/mL | 150 ng/mL |
| Boric Acid | 1000 | Neg | Neg | Neg |

**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 a norketamine 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 Norketamine Concentration | | |
|---|---|---|---|
| | 0 ng/mL | 75 ng/mL | 125 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 1.003 to 1.025 were split into three portions each and either left un-spiked or spiked to a norketamine concentration of either 75 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.

| Specific Gravity | Spiked Norketamine Concentration | | |
|---|---|---|---|
| | 0 ng/mL | 75 ng/mL | 125 ng/mL |
| 1.003 | Neg | Neg | Pos |
| 1.005 | Neg | Neg | Pos |
| 1.008 | Neg | Neg | Pos |
| 1.010 | Neg | Neg | Pos |
| 1.015 | Neg | Neg | Pos |
| 1.018 | Neg | Neg | Pos |
| 1.020 | Neg | Neg | Pos |
| 1.022 | Neg | Neg | Pos |
| 1.025 | Neg | Neg | Pos |

## Symbols Used

| | | | |
|---|---|---|---|
| EC REP | Authorized Representative | REAGENT 1 | $R_1$, Antibody/ Substrate Reagent |
|  | Biological Risks | REAGENT 2 | $R_2$, Enzyme-Drug Conjugate Reagent |
| CE | CE Mark | REF | Reference Number |
|  | Consult Instructions for Use | SDS | Safety Data Sheet |
| CONTENTS | Contents |  | Temperature Limits |
| LOT | Lot Number |  | Use-by Date |
|  | Manufacturer | | |

## Bibliography


1. Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **23**(82):7920-7970 (2017).
3. Bergman, S.A., Ketamine: review of its pharmacology and its use in pediatric anesthesia. *Anesth Prog* **46**:10-20 (1999).
4. Brau, M.E., Sander, F., Vogel, W., and Hempelmann, G., Blocking mechanisms of ketamine and its enantiomers in enzymatically demyelinated peripheral nerve as revealed by single-channel experiments. *Anesthesiology*. **86**(2):394–404 (1997).
5. Reich, D.L. and Silvay, G., Ketamine: an update on the first twenty-five years of clinical experience. *Can J Anaesth* **36**:186-97 (1989).
6. White, J.M. and Ryan, C.F., Pharmacological properties of ketamine. *Drug Alc Review* **15**:145-155 (1996).
7. World Health Organization, 37th Expert Committee on Drug Depencence,ECDD Agenda Item 6.1 (2015).
8. Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., et al. Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry.* **47**(4):351-4 (2000).
9. Zarate Jr, C.A., Singh, J.B., Carlson, P.J., et al. A randomized trial of an n-methyl-d-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*. **63**(8):856-64 (2006).
10. Jansen, K.L. and Darracot-Cankovic, R. The nonmedical use of ketamine, part two: A review of problem use and dependence. *J Psychoactive Drugs.* **33**:151–158 (2001).


## Bibliography, continued


11. Moore, K.A., Kilbane, E.M., and Jones, R. Tissue distribution of ketamine in a mixed drug fatality. *J. Forensic Sci.* **42(6):** 1183–1185 (2007).
12. Moreton, J.E., Meisch, R.A., Stark, K., et al. Ketamine self-administration by the rhesus monkey. *J. Pharmacol. Exp. Ther.* **203:** 303-309 (1977).
13. Lua, A.C., Lin, H.R., Tseng, Y.T., Hu, A.R., and Yeh, P.C. Profiles of urine samples from participants at rave party in Taiwan: prevalence of ketamine and MDMA abuse. *Forensic Sci. Int.* **36:** 47–51(2003).
14. Curran, H.V. and Morgan, C. Cognitive, dissociative and psychogenic effects of ketamine in recreational users on the night of drug use and 3 days later. *Addiction* **95(4):**575–590 (2000).
15. Degenhardt, L., Copeland, J., and Dillon, P. Recent trends in the use of "club drugs": an Australian review. *Subst Use Misuse*. **40**(9–10): 1241–1256 (2005).
16. Hijazi, Y. and Bolieu, R.. Contribution of CYP3A4, CYP2B6 and CYP2C9 isoforms to N-methylation of ketamine in human liver microsomes. *Drug Metab. Dispos.* **30:** 853–858 (2002).
17. Leung, L.Y. and Baillie, T.A. Comparative pharmacology in the rat of ketamine and its two principal metabolites, norketamine and (Z)-6-hydroxynorketamine. *J. Med. Chem.* **29**:2396-2399 (1986)
18. Wieber, J., Gugler, R., Hengstmann, J.H., and Dengler, H.J. Pharmacokinetics of ketamine in man. *Anaesthesist* **24**:260-263 (1975).
19. Harun, N., Anderson, R.A., and Miller,E.I. Validation of an Enzyme-Linked Immunosorbent Assay Screening Method and a Liquid Chromatography–Tandem Mass Spectrometry Confirmation Method for the Identification and Quantification of Ketamine and Norketamine in Urine Samples from Malaysia. *J Anal Toxicol.* **33**:310-321 (2009).
20. Karch, S.B. and Drummer, O.H. Karch's pathology of drug abuse. 5th ed. Boca Raton (FL): CRC Press, Taylor & Francis Group (2016).
21. Adamowicz, P. and Kala, M. Urinary excretion rates of ketamine and norketamine following therapeutic ketamine administration: method and detection window considerations. *J Anal Toxicol*.**29**:376–382 (2005).
22. Zhen, L. Effects of filtration sterilization on the stability of ketamine, selected benzodiazepines and metabolites in female urine. Boston University Theses & Dissertations (2017). OpenBU: https://open.bu.edu/handle/2144/20791
23. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**:46 (1972).
24. Sodium Azide National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html.


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