LZI Phencyclidine Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0010 (100/37.5 mL R_1/R_2 Kit)
0011 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Phencyclidine (PCP) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of PCP in human urine at a cutoff value of 25 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

Phencyclidine (PCP), or Angel Dust, is a potent hallucinogen and surgical anesthetic. It was formerly used in medical applications, but is no longer in clinical use due to its undesirable side effects (3). PCP and its analogs, however, are frequently mixed with marijuana or tobacco and smoked. Adverse abuse of PCP results in conditions such as tachycardia, psychosis, paranoia, and mydriasis. It can result in death in case of overdose (4, 5).

Assay Principle

The PCP assay is a homogeneous enzyme immunoassay with 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 (6). 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, PCP-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 the free drug; the unbound PCP-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-phencyclidine 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 phencyclidine 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.*

PHENCYCLIDINE Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 12.5 ng/mL phencyclidine	0012
Cutoff Calibrator: Contains 25 ng/mL phencyclidine	0013
Intermediate Calibrator: Contains 50 ng/mL phencyclidine	0014
High Calibrator: Contains 100 ng/mL phencyclidine	0015

PHENCYCLIDINE Controls	REF
Level 1 Control: Contains 18 ng/mL phencyclidine	0017
Level 2 Control: Contains 32 ng/mL phencyclidine	0018

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 (7).*
- *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 sample may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (8). 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 (9, 10). For longer storage, up to 12 months, keep sample frozen at -20ºC and then thaw before use (11, 12). 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 Hitachi 717 and the Synermed IR500 clinical analyzers.

Assay Procedure

Refer to the specific parameter used for each analyzer before performing the assay. For qualitative analysis, use the 25 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: 18 ng/mL and 32 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 quality 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 25 ng/mL of PCP, 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 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 PCP 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 phencyclidine. 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 urine test results.*
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 performed with a single Hitachi 717 automated 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	168.0	0.7	0.4 %	168.0	0.9	0.5 %
18 ng/mL	238.6	1.2	0.5 %	238.8	0.6	0.2 %
25 ng/mL	264.6	1.2	0.5 %	264.6	0.8	0.3 %
32 ng/mL	282.8	1.4	0.5 %	284.1	0.9	0.3 %
100 ng/mL	341.2	0.7	0.2 %	340.1	1.1	0.3 %

**Run-to-Run 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
18 ng/mL	17.4	0.3	1.5 %	17.1	0.4	2.5 %
25 ng/mL	24.7	0.4	1.5 %	24.3	0.6	2.3 %
32 ng/mL	31.6	0.5	1.7 %	31.4	0.6	1.9 %

**Run-to-Run 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 1 ng/mL.

Accuracy: One hundred and forty one (141) urine samples from healthy, non-user volunteers were tested for PCP with the assay. All specimens were found negative. In addition, 158 clinical urine specimens were also tested with both a commercially available EIA and the LZI PCP Enzyme Immunoassay. Fifty (50) samples were found positive and 108 samples were found negative by both assays.

Cutoff Value (25 ng/mL)	Commercial Kit	LZI PCP EIA	% Agreement with Predicate
# Positive Samples	50	50	100 %
# Negative Samples	108	108	100 %
Total # of Samples	158	158	N/A

Finally, 14 clinical samples (including four diluted from higher concentration ones) with PCP concentration near cutoff (15 ng/mL to 38 ng/mL by GC/MS) were evaluated. All samples with GC/MS values ≥ 25 ng/mL (9) tested positive, and those < 25 ng/mL (5) tested negative by the current EIA.

Analytical Recovery: In qualitative analysis, the assay correctly identified spiked samples containing more than 25 ng/mL of PCP (n=25, spiked levels equal or higher than Level 2 Control) as positive, and those containing less than 25 ng/mL of PCP (n=25, spiked levels equal to or less than Level 1 Control) as negative. For semi-quantitative analysis, the average recovery for samples (five samples at each level) spiked with 4 ng/mL to 90 ng/mL of PCP is summarized in the following table:

Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
4	4.7	117.5 %
8	8.4	104.8 %
12	11.9	99.0 %
16	15.6	97.3 %
18	17.3	96.1 %
32	31.3	97.8 %
40	37.9	94.7 %
60	55.9	93.2 %
80	73.0	91.3 %
90	77.7	86.3 %

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 Phencyclidine Compounds:

Compound	Target [ ] (ng/mL)	% Cross-Reactivity
Phencyclidine	25	Positive

Structurally Unrelated Pharmacological Compounds:

Compound	Target [ ] (µg/mL)	% Cross-Reactivity
Acetaminophen	1000	Negative
Acetylsalicyclic Acid	1000	Negative
Amobarbital	1000	Negative
Amphetamine	1000	Negative
Benzoylecgonine	3000	Negative
Bromopheniramine	100	Negative
Bupropion	100	Negative
Caffeine	100	Negative
Chlorpheniramine	50	Negative
Chlorpromazine	100	Negative
Codeine	100	Negative
Dextromethorphan	1000	Negative
Diphenhydramine	100	Negative
Ephedrine	1000	Negative
Ketamine	100	Negative
Meperidine	100	Negative
Methadone	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	100	Negative
Morphine	500	Negative
Naloxone	1000	Negative
Naltrexone	25	Negative
Nicotine	1000	Negative
Norpropoxyphene	100	Negative
Nortriptyline	100	Negative
Oxazepam	1000	Negative
Phenobarbital	1000	Negative
Phenylpropanolamine	1000	Negative
Primidone	1000	Negative
Promethazine	100	Negative
Propranolol	100	Negative
Propoxyphene	1000	Negative
Pseudoephedrine	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Thioridazine	1000	Negative
Triprolidine	150	Negative
Tyramine	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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Authorized European Rep. within the EU:
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Printed in USA