LZI Cotinine Enzyme Immunoassay – EU Only

IVD For In Vitro Diagnostic Use Only

REF 0480 (100/37.5 mL R_1/R_2 Kit)
0481 (1000/375 mL R_1/R_2 Kit) 2-8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Cotinine Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cotinine in human urine at a cutoff value of 500 ng/mL. The assay is designed for 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 immunoassay result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Nicotine is the primarily addictive compound in tobacco products (3). For the past three decades, there has been a tremendous attention to tobacco smoking and the so-called "passive inhalation" of tobacco smoke due to its correlation to lung cancer.

Inhaled tobacco smoke reaches small airways and alveoli of the lungs, where 90 % of nicotine is absorbed. When nicotine is absorbed, it is readily metabolized into cotinine by the liver (4). Urine concentrations of both nicotine and cotinine correlate with cigarette use in active smokers (5). While nicotine has a very short half -life of approximately 40 minutes (6), cotinine has an average half-life of 20 hours (7), and can be detected in the urine of a smoker even several days after the smoking has ceased.

Several methods have been used to determine the smoking status of an individual. These include measurement of thiocyanate, carbon monoxide, and cotinine. Measurement of both thiocyanate and carbon monoxide, however, is more likely to be affected by environmental factors and can cause false positive results. Since cotinine can only be derived from metabolism of nicotine, it is a better marker for determination of the smoker status.

Assay Principle

The LZI Cotinine 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 (8). 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, cotinine-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 cotinine-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-cotinine 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 cotinine 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.*

COTININE Calibrators/Controls	REF
Negative Calibrator	0001
Low Calibrator/Level 1 Control: 250 ng/mL cotinine	0482
Cutoff Calibrator: 500 ng/mL cotinine	0483
Intermediate Calibrator: 1000 ng/mL cotinine	0484
High Calibrator: 2000 ng/mL cotinine	0485
Level 2 Control: 750 ng/mL cotinine	0488

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 (9).*
- *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

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (10). 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 (11, 12). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown cotinine analytes in urine are stable at -20ºC for up to seven weeks (12, 13). Samples should be at room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis. Urine samples within the normal pH range of 5-8 can be tested without any pretreatment. Fresh and properly stored urine sample are generally within this range (14).

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. If other instruments are used, performance will need to be validated by the laboratory (15, 16).

Assay Procedure

Analyzers with the 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 the Hitachi 717 analyzer include a 20 µL sample, 200 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) at 37ºC incubation temperature, 26-30 reading points, and a 340 nm primary wavelength. For qualitative analysis, use 500 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 reference point: use the 250 ng/mL and 750 ng/mL for the 500 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 guidelines and regulations.

Results

Note: A positive test result does not necessarily mean a person is a smoker and a negative test result does not necessarily mean a person did smoke. There are a number of factors that influence the reliability of immunoassays.

Qualitative: The urine concentration of cotinine for non-smokers is normally well below 500 ng/mL (4, 5). The urine concentration of cotinine for active smoker averages from 1000 ng/mL to as high as 8000 ng/mL (5). 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 calibrator used as the cutoff 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 cotinine 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 cotinine. The test is not intended for quantifying this single analyte in samples.*
2. *A preliminary positive result does not necessarily indicate a person is a smoker.*
3. *A negative result does not necessarily mean a person did not smoke.*
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 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
Negative	414.6	2.2	0.5 %	412.1	2.9	0.7 %
250 ng/mL	456.6	3.5	0.8 %	453.1	4.1	0.9 %
500 ng/mL	483.6	3.2	0.7 %	483.4	2.6	0.5 %
750 ng/mL	508.8	2.8	0.6 %	507.5	2.1	0.4 %
2000 ng/mL	593.4	3.9	0.7 %	589.1	5.4	0.9 %

** Run-to-Run testing completed over 2 weeks.*

Semi-quantitative analysis: The concentrations of 500 ng/mL and the two levels of controls were determined with reference curve 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
250 ng/mL	252.8	9.5	3.8 %	256.0	14.9	5.8 %
500 ng/mL	505.7	13.3	2.6 %	503.9	27.8	5.5 %
750 ng/mL	755.4	17.1	2.3 %	742.5	17.6	2.4 %

** Run-to-Run testing completed over 2 weeks.*

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from negative urine with 95 % confidence, was tested to be 25 ng/mL.

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine to various concentrations and evaluated with the assay's calibrated dose response curve.

The following table summarizes the approximate quantity of each compound that is equivalent in assay reactivity to the 500 ng/mL cotinine cutoff (positive) or the maximal concentration of the compound tested that gave a response with cross-reactivity below the response of the cutoff calibrator (positive).

Structurally Related Cotinine Compounds*:

Compound	Test [ ] (ng/mL)	Result [ ] (ng/mL)	% Cross-Reactivity
(R,S)-Norcotinine	62,500	571.7	0.91%
S(-)-Nicotine	250,000	204.2	0.08%
(+)-Anabasine	250,000	22.3	0.01%
(+/-)-Nornicotine	250,000	35.8	0.01%
trans-3'-hydroxycotinine	15,625	853.5	5.46%

Structurally Unrelated Compounds:

Compound	Test [ ] (ng/mL)	Cross-Reactivity		
		Spiked Cotinine Concentration		
		0 ng/mL (ng/mL)	(-25% Cutoff) 375 ng/mL Control (ng/mL)	(+25% Cutoff) 625 ng/mL Control (ng/mL)
Acetaminophen	35,000	Neg	Neg	Pos
6-Acetylmorphine	10,000	Neg	Neg	Pos
Acetylsalicylic Acid	35,000	Neg	Neg	Pos
Albuterol (Salbutamol)	35,000	Neg	Neg	Pos
Amitriptyline	35,000	Neg	Neg	Pos
d-Amphetamine	100,000	Neg	Neg	Pos
Benzoylecgonine	35,000	Neg	Neg	Pos
Buprenorphine	15,000	Neg	Neg	Pos
Bupropion	35,000	Neg	Neg	Pos
Caffeine	35,000	Neg	Neg	Pos
Carbamazepine	50,000	Neg	Neg	Pos
Cetirizine	35,000	Neg	Neg	Pos
Chlorpheniramine	35,000	Neg	Neg	Pos
Chlorpromazine	35,000	Neg	Neg	Pos
Clomipramine	100,000	Neg	Neg	Pos
Codeine	100,000	Neg	Neg	Pos
Desipramine	35,000	Neg	Neg	Pos
Diphenhydramine	100,000	Neg	Neg	Pos
Ephedrine	100,000	Neg	Neg	Pos
Fentanyl	10,000	Neg	Neg	Pos
Fluoxetine	35,000	Neg	Neg	Pos
Fluphenazine	50,000	Neg	Neg	Pos
Hydrocodone	35,000	Neg	Neg	Pos
Hydromorphone	50,000	Neg	Neg	Pos
Ibuprofen	35,000	Neg	Neg	Pos
Imipramine	35,000	Neg	Neg	Pos
Lidocaine	35,000	Neg	Neg	Pos
Loratidine	50,000	Neg	Neg	Pos
Maprotiline	50,000	Neg	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	Neg	Neg	Pos
MDEA	100,000	Neg	Neg	Pos
MDMA (3,4-methylenedioxyamphetamine)	100,000	Neg	Neg	Pos
Meperidine	100,000	Neg	Neg	Pos
Methadone	35,000	Neg	Neg	Pos
d-Methamphetamine	100,000	Neg	Neg	Pos
Methapyrilene	35,000	Neg	Neg	Pos
Methaqualone	35,000	Neg	Neg	Pos
Metronidazole	35,000	Neg	Neg	Pos
Morphine	35,000	Neg	Neg	Pos
Nicotine	100,000	Neg	Neg	Pos
Nortriptyline	35,000	Neg	Neg	Pos
Oxazepam	100,000	Neg	Neg	Pos
Oxycodone	35,000	Neg	Neg	Pos
Oxymorphone	100,000	Neg	Neg	Pos
PCP (phencyclidine)	10,000	Neg	Neg	Pos
Pentazocine	20,000	Neg	Neg	Pos
Phenobarbital	100,000	Neg	Neg	Pos
d-Propoxyphene (Dextropropoxyphene)	35,000	Neg	Neg	Pos
Propranolol	35,000	Neg	Neg	Pos
Ranitidine	50,000	Neg	Neg	Pos
Sertraline	35,000	Neg	Neg	Pos
THC-COOH (11-Nor-Delta-9-THC-9-carboxylic acid)	1000	Neg	Neg	Pos
Thioridazine	50,000	Neg	Neg	Pos
Tramadol	50,000	Neg	Pos	Pos
Valproic Acid	50,000	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.

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, **53**(69): 11970 (1988).
3. Surgeon General of the United States. Nicotine Addiction: The health consequences of smoking. Washington, D.C. US Department of Helath and Human Services, (1998).
4. Benoweitz, N.L., The human pharmacology of nicotine, *Res Adv Alcohol Drug Probl*, **9**:1 (1986).
5. Benowitz, N.L., and Jacob III, P., Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers, *Clin Pharmacol Ther*, **53**:316 (1993).

Bibliography, continued:

6. Javis, M.J., Tunstall-Pedoe, H., Feyerabend, C., and Vesey, C., Comparison of tests used to distinguish smokers from non-smokers, *AM. J. Public Health*, **77**:1435 (1987).
7. Moyer, T.P., Charlson, J.R., Enger, R.J., Dale, L.C., Ebbert, J.O., Schroeder, D. R., and Hurt, R.D., Simultaneous analysis of Nicotine, Nicotine Metabolites, and Tobacco Alkaloids in Serum or Urine by Tandom Mass Spectrometry, with Clinical Relevant Metabolic Profiles, *Clin Chem*, **48**:1460 (2002).
8. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**:46 (1972).
9. 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
10. Yahya, A.M., McElnay, J.C., and D'Arcy, P.F., Drug absorption to glass and plastics, *Drug Metabol Drug Interact*, **6**(1):1-45 (1988).
11. Moyer, T. P., Charlson, J. R., Enger, R. J., Dale, L. C., Ebbert, J. O., Schroeder, D. R., and Hurt, R. D., Simultaneous Analysis of Nicotine, Nicotine Metabolites, and Tobacco Alkaloids in Serum or Urine by Tandem Mass Spectrometry, with Clinically Relevant Metabolic Profiles, Clin Chem. 48(9):1460-71 (2002).
12. Riboli, E., Haley, N. J., De Waard, F., and Saracci, R., Validity of Urinary Biomarkers of Exposure to Tobacco Smoke following Prolonged Storage, International Journal of Epidemiology, 24(2): 354-358 (1995).
13. McGuffey, J. E., Wei, B., Bernert, J. T., Morrow, J. C., Xia, B., Wang, L., and Blount, B. C., Validation of a LC-MS/MS Method for Quantifying Urinary Nicotine, Six Nicotine Metabolites and the Minor Tobacco Alkaloids—Anatabine and Anabasine—in Smokers' Urine, PLOS One, 9(7): 1-13 (2014).
14. Pitts, R.F., and Alexander, R.S., The nature of the renal tubular mechanism for acidifying the urine, *Am J Physiol*, **144**:239–254 (1945).
15. Nichols, J., Instrument Validation: The Road to Success. CLN's Lab 2004: From Basic to Advanced Series. 14-16 (2004).
16. CDRH Guidance for Industry and FDA Staff: Replacement Reagent and Instrument Family Policy (2003).

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