LZI Oral Fluid Cotinine II Enzyme Immunoassay

REF S0520 (75/37.5 mL R_1/R_2 Kit)
S0521 (750/375 mL R_1/R_2 Kit)

2ºC 8ºC

Lin-Zhi International, Inc. **For Forensic Use Only**

Intended Use

The LZI Oral Fluid Cotinine II Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of cotinine in neat human oral fluid, collected into an LZI Oral Fluid Collector, at the cutoff value of 25 ng/mL when calibrated against cotinine. The assay is intended as an aid in the detection of cotinine after use or exposure to tobacco products. 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 confirmation 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 method (e.g., gas or liquid chromatography and mass spectrometry) must be used in order 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

Nicotine is the primarily addictive compound in tobacco products (3). For the past three decades, there has been 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 Oral Fluid Cotinine II 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 would bind to 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 340 nm primary wavelength.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains a 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*

Materials required (but not provided)

**Calibrators and Controls are sold separately and contain negative synthetic oral fluid matrix with sodium azide as a preservative.*

ORAL FLUID COTININE II Calibrator/Control	REF #
Oral Fluid Negative Calibrator	S0001
Low Calibrator: Contains 12.5 ng/mL cotinine	S0522
Cutoff Calibrator: Contains 25 ng/mL cotinine	S0523
Intermediate Calibrator: Contains 50 ng/mL cotinine	S0524
High Calibrator: Contains 75 ng/mL cotinine	S0525
Level 1 Control: Contains 18.75 ng/mL cotinine	S0526
Level 2 Control: Contains 31.25 ng/mL cotinine	S0527

Collectors**

***Collectors are sold separately.*

ORAL FLUID Collectors	REF #
LZI Oral Fluid Collector –50 mL Polypropylene Centrifuge Tube	S0000b

Precautions and Warning

- *This test is for Forensic Use Only. This test should not be repackaged for in vitro diagnostic use.*
- *Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing of 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 stored refrigerated at 2-8ºC when not in use. See the expiration date on individual bottle labels.

Specimen Storage and Shipping

Note: If oral fluid samples cannot be analyzed immediately, they may be stored refrigerated (2-8ºC) for up to 21 days or frozen (-20ºC) for up to 21 days (10). Studies have been performed for up to 21 days to show cotinine is stable in oral fluid. No further study was conducted beyond 21 days.

Samples should always be shipped cold (2-8ºC), packed in gel ice, and shipped for next-day delivery (within 24 hours). Failure to store or ship samples under these conditions may result in a significant decrease in the recovery of the analyte. Please see additional details in the Specimen Collection and Handling section below.

Specimen Collection and Handling

Oral fluid samples should be collected into a device without an absorbing pad, such as the LZI Oral Fluid Collector (a 50 mL polypropylene centrifuge tube).

Prior to testing, samples should be frozen overnight (at minimum) and then allowed to thaw at room temperature. Samples should then be spun for five minutes at 3000 rpm to remove particulates. Only the clear top layer should be assayed for EIA testing and/or confirmatory testing. Samples should be at room temperature (18-25ºC) for testing.

Samples do not require dilution or any additional correction factors.

Fresh and properly stored oral fluid samples should be within the normal pH range of 6-8; however, any sample with pH ranging from 3-10 can be tested without any pretreatment of the samples.

Handle all oral fluid 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.

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.
For qualitative analysis, use the 25 ng/mL Cutoff calibrator. For semi-quantitative analysis, use all five calibrators and controls. Recalibration should be performed after a reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 18.75 ng/mL and 31.25 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 changes 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, guidelines, and regulations.

Results

Note: A positive test result does not always mean a person smoked and a negative test result does not always mean a person did not smoke. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 25 ng/mL of cotinine, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (mAU) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (mAU) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for the 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 drug abuse.*
3. *A negative result does not necessarily mean a person did not smoke.*
4. *There is a possibility that other substances and/or factors not listed above may interfere with the test and cause incorrect results (e.g., technical or procedural error, fluid intake, endogenous or exogenous interferents).*
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 oral fluid only.*
7. *The test is not for therapeutic drug monitoring.*

Symbols

LZI uses the symbols and signs listed on the symbol glossary on the website. Visit www.lin-zhi.com/symbol-glossary for detailed information.

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

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