LZI Oral Fluid Propoxyphene Enzyme Immunoassay

REF S0120c (75/37.5 mL R_1/R_2 Kit)
S0121c (750/375 mL R_1/R_2 Kit) 2ºC 8ºC

FOR RESEARCH & DEVELOPMENT USE ONLY

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Oral Fluid Propoxyphene Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of propoxyphene in neat human oral fluid, collected into an LZI Oral Fluid Collector, at the cutoff value of 20 ng/mL. The assay is designed for prescription use with a number of automated clinical chemistry analyzers. This is a Non-FDA Approved assay for Research & Development use only and as such should not be repackaged for in vitro diagnostic use.

The assay provides a rapid screening procedure for determining the presence of propoxyphene in human oral fluid. 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) is the preferred confirmatory method (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

Propoxyphene, or dextropropoxyphene, the active principle in the prescription drug Darvon, is one of the most prescribed pain relievers for mild to moderate discomfort. Chemically, it resembles methadone, and is used as an alternative for detoxification and maintenance of narcotic dependence (3). However, propoxyphene and its metabolites are cardiotoxic, so the likelihood of overdose is higher than with methadone. Overdosage can result in convulsions, respiratory depression, cardiac arrhythmia, hypertension, pulmonary edema, circulatory collapse, and death (4-6). Propoxyphene is primarily metabolized to norpropoxyphene via N-demethylation. Norpropoxyphene is a biologically active analgesic as well; however, it is less potent than propoxyphene. Further demethylation and dehydration lead to a cyclic dinorpropoxyphene metabolite. Other polar metabolites are formed by aryl hydroxylation, ester hydrolysis, and glucuronide conjugation (7).
The rate of clearance varies from person to person; however, approximately 34 % of the administered dosage is eliminated within 20 hours (7, 8), and up to 75 % is secreted in the urine over a seven day period (9). Detection of propoxyphene or its metabolites in oral fluid indicates use of propoxyphene.

Assay Principle

The LZI Oral Fluid Propoxyphene Enzyme Immunoassay is a homogeneous enzyme immunoassay with ready-to-use liquid reagents. 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 (10). 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, propoxyphene-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 free drug, and the unbound propoxyphene-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 mouse monoclonal anti-propoxyphene 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 propoxyphene-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and Controls are sold separately and contain negative synthetic oral fluid matrix with Proclin 300 ™ as a preservative.*

ORAL FLUID PROPOXYPHENE (PPX) Calibrator/Control	REF #
Oral Fluid LIS Negative Calibrator	S0008
Cutoff Calibrator: Contains 20 ng/mL propoxyphene	S0123c
High Calibrator: Contains 50 ng/mL propoxyphene	S0125c
Low Calibrator / Level 1 Control: Contains 10 ng/mL propoxyphene	S0127c
Intermediate Calibrator / Level 2 Control: Contains 30 ng/mL propoxyphene	S0128c

Collectors **

***Collectors are sold separately.*

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

Precautions and Warning

- *This test is a Non-FDA approved assay and is for Research & Development 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 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 (8/16/76).*
- *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 Storage and Shipping

Note: If oral fluid samples cannot be analyzed immediately, they may be stored in amber glass vials and refrigerated (2-8ºC) for up to 21 days or frozen (-20ºC) for up to 21 days. Studies have been performed up to 21 days to show propoxyphene 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 recovery of analyte.

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) (11).
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 enzyme 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.

Assay Procedure

Analyzers with 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 48 µL sample, 150 µL of antibody reagent (R_1), and 75 µL of enzyme conjugate reagent (R_2) in 37ºC incubation temperature, 30-35 reading frames, and 340 nm primary wavelength.
For qualitative analysis, use the 20 ng/mL cutoff calibrator. For semi-quantitative analysis, use all five calibrators and controls. 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: 10 ng/mL and 30 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, guidelines, and regulations.

Results

Note: A positive test result does not always mean a patient took illegal drugs and a negative test result does not always mean a patient did not take illegal drugs. There are number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 20 ng/mL of propoxyphene, is used as a reference for distinguishing 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 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 a confirmatory method such as GC/MS or LC/MS, or (2) permitting laboratories to establish quality control procedures.
This mode requires a calibration curve that can be established with the five assay calibrators and controls.

Limitations

1. *A positive result from the assay indicates only the presence of propoxyphene. The test is not intended for quantifying this single analyte in samples.*
2. *A positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take propoxyphene.*
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, endogeneous or exogeneous interferents).*
5. *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.*

Bibliography:

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11. LZI Oral Fluid Sample Preparation Sheet.

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