MEDICA

IVD CE

REF 14248-4 **4 x 22 mL/9 mL**

PROPOXYPHENE - Qualitative (PPX)
Wedges each contain usable volumes of 22 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE
The EasyRA propoxyphene (PPX) reagent is intended for the qualitative determination of propoxyphene in human urine at a cutoff value of 300 ng/mL. The assay is designed for prescription use only on the EasyRA Clinical Chemistry Analyzer. For *in-vitro* diagnostic use only.

The assay provides a rapid screening procedure for determining the presence of propoxyphene in urine. The assay provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography/mass spectrometry (GC/MS) or Liquid Chromatography/mass spectrometry (LC/MS) are the preferred confirmatory methods.[1, 2] Clinical consideration and professional judgment should be exercised to any drug of abuse test result, particularly when the preliminary test result is positive.

SUMMARY AND EXPLANATION
Propoxyphene, or dextropropoxyphene, the active ingredient in the prescription drug Darvon, has been 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 cardio toxic, the likelihood of overdose is higher than methadone. Over dosage can result in convulsions, respiratory depression, cardiac arrhythmia, hypertension, pulmonary edema, circulatory collapse and death.[4, 5, 6]

Propoxyphene is primarily metabolized to norpropoxyphene via N-demethylation. Norpropoxyphene is a biologically active analgesic as well, however, less potent than propoxyphene. Further demethylation and dehydration lead to 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 hr[7, 8] , and up to 75% is secreted in the urine over a 7 day period[9] have been reported.

Detection of propoxyphene or its metabolites in urine indicates use of propoxyphene.

PRINCIPLE OF THE PROCEDURE
The propoxyphene assay is a homogeneous enzyme immunoassay[10] which provides qualitative results relative to a single calibration cutoff value. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenate (G6PDH) for a fixed amount of antibody in the reagent. 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 free drug is present in the sample, antibody will bind 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 increase that can be measured spectrophotometrically at 340 nm.

REAGENTS

Antibody/Substrate Reagent (R1): Contains 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 (R2): Contains propoxyphene-labeled glucose-6-phosphate dehydrogenase (G6PDH), buffer and sodium azide (0.09 %) as a preservative.

Precautions

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagent contains less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Material Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

Instructions for Reagent Handling, Storage and Stability

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date on the label if stored at 2-8 ^{o}C. The reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of day programmed on the RFID chip on the reagent wedge. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area.

Specimen Collection and Storage / Stability

Urine sample may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended[11]. Use fresh urine specimen for the test. If the sample cannot be analyzed immediately, it may be stored refrigerated at 2-8°C for up to 3 days. For longer storage keep sample frozen at -20°C and thaw before use. Studies have shown propoxyphene analytes in urine are stable at -20ºC for up to 6 months[12]. Samples should be brought to a room temperature of 18-25 ^{o}C 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 both samples should be forwarded to the laboratory for testing. Handle all urine specimens as if they are potentially infectious.

PROCEDURE

Materials Provided:

Medica PPX Reagent Wedge, REF 14248 (Qualitative)

Additional materials required:

Medica EasyCal Propoxyphene Cutoff Calibrator (Propoxyphene Cutoff, 300 ng/mL), REF 14684
Medica EasyQC Propoxyphene Negative Control (Propoxyphene, 225 ng/mL), REF 14786
Medica EasyQC Propoxyphene Positive Control (Propoxyphene, 375 ng/mL), REF 14787
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or
Medica Cleaner Wedge – Chemistry, REF 10661
Medica EasyRA Evaporation Caps, REF 10745

INSTRUCTIONS FOR USE

The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray in the reagent area. Dry the neck of the reagent wedge and check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2. Place Medica EasyRA Evaporation Caps, REF 10745 on both the R1 and R2 openings of the reagent wedge.

NOTE: Use of the Medica EasyRA Evaporation Cap is required to guarantee on-board calibration stability.

Calibration

Medica EasyCal Propoxyphene cut-off Calibrator, REF 14684 is required for the calibration of the qualitative assay. The calibration interval (20 days maximum) with Evaporation Caps is programmed on the RFID chip on the reagent wedge Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of human urine-based controls (positive and negative) be run with the assay at least once every day and with each reagent lot change. Failure to obtain the proper values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should also follow local, state, and federal quality control guidelines when using quality control materials.

Results
The cutoff calibrator, which contains 300 ng/mL of propoxyphene, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit time (mA/min) that is equal to, or greater than, that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance value per unit time lower than that obtained with the cutoff calibrator is considered negative.

Procedural Limitations
1. The test is not intended for quantifying these single analytes 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. 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. Positive results should be confirmed by other affirmative, analytical chemical methods (e.g., chromatography), preferably GC/MS or LC/MS.

The test is designed for use with human urine only.PERFORMANCE CHARACTERISTICS

The results shown below were obtained with the EasyRA analyzer. .

Inaccuracy/Correlation

One hundred and eight (108) clinical urine specimens were tested qualitatively with the Enzymatic Immunoassay (EIA) method on the EasyRA. . All results were confirmed with LC/MS* and are summarized in the table below:

	(<150 ng/mL) Negative	**Near Cutoff (150-300 ng/mL) Negative**	**Near Cutoff (300-450 ng/mL) Positive**	**(>450 ng/mL) Positive**
EasyRA	**LC/MS**	**LC/MS**	**LC/MS**	**LC/MS**
Positive (>300 ng/ml)	0	2	10	45
Negative (<300 ng/ml)	54	7	0	0
% Agreement Negative	**96.80%**			
% Agreement Positive	**100%**			

***** LC/MS data represents the total of propoxyphene plus norpropoxyphene

Imprecision (CLSI, EP5-A2)

Qualitative analysis: Nine samples of Propoxyphene spread evenly throughout the range of 0-600 ng/mL were prepared in human urine and analyzed in duplicate twice a day for 20 days. The samples were tested in qualitative mode and the absorbance change versus time was also measured for each reading. Typical results (mA/min) are as follows:

Within Run Imprecision (EP5-A2)
Qualitative Results (n = 80)

Samples (ng/ml)	**Mean (mA/Min)**	**SD (mA/Min)**	**%CV**
0	0.0611	0.0008	1.29
75	0.0653	0.0006	0.89
150	0.0731	0.0008	1.11
225	0.0865	0.0012	1.38
300	0.1041	0.0009	0.91
375	0.1197	0.0011	0.90
450	0.1290	0.0010	0.80
525	0.1357	0.0011	0.76
600	0.1423	0.0011	0.75

Total Imprecision (EP5-A2)
Qualitative Results (n = 80)

Samples (ng/ml)	**Mean (mA/Min)**	**SD (mA/Min)**	**%CV**
0	0.0611	0.0008	1.35
75	0.0653	0.0007	1.06
150	0.0731	0.0010	1.44
225	0.0865	0.0017	1.99
300	0.1041	0.0019	1.84
375	0.1197	0.0016	1.37
450	0.1290	0.0014	1.09
525	0.1357	0.0020	1.38
600	0.1423	0.0013	0.93

% Agreement of Qualitative Precision Results with Target Values

Samples (ng/mL)	Number Positive	Number Negative	% Agreement
0	0	80	100%
75	0	80	100%
150	0	80	100%
225	0	80	100%
300	18	62	N/A
375	80	0	100%
450	80	0	100%
525	80	0	100%
600	80	0	100%

Specificity

Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix and both QC levels to various concentrations and evaluated against the cutoff calibrator. The table listed 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).

Cross-Reactant	Concentration (ng/mL)	Cross-reactivity
Propoxyphene	>300	Positive
Norpropoxyphene	>700	Positive
	Concentration (µg/mL)	
Acetaminophen	1000	Negative
Acetylsalicylic acid	1000	Negative
Amitriptyline	820	Negative
Amphetamine	960	Negative
Benzoylecgomine	930	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine		Negative
Codeine	1280	Negative
Dextromethorphan	1000	Negative
Ecgonine	100	Negative
Ephedrine	1000	Negative
Imipramine	700	Negative
Lidocaine	1000	Negative
Meperidine	740	Negative
Methadone	970	Negative
Methamphetamine	960	Negative
Methaqualone	640	Negative
Morphine	1290	Negative
Nortriptyline	780	Negative

Phencyclidine	680	Negative
Phenobarbital	700	Negative
Promethazine	1000	Negative
Ranitidine	1000	Negative
Secobarbital	680	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.

Endogenous Interferents (For Reference purposes only)

Endogenous Metabolites evaluated at 300 ng/ml Propoxyphene*

Compound	**Level Added mg/dL**	**Interference***
Conjugated Bilirubin	10	No
Ascorbic Acid	>500	No
Creatinine	500	No
Glucose	1200	No
Hemoglobin	100	No
Albumin	300	No
Oxalic Acid	>150	No
Sodium Chloride	2300	No
Acetone	790	No
Ethanol	790	No
pH	Adjust to pH 4.5 and 8.0	No
Specific Gravity	Range Tested 1.005 to 1.031	No

*Interference is positive if added substance changes Propoxyphene values at 300ng/mL by more than 10%; pH and specific gravity tested at 225 ng/mL, 300ng/mL and 375 ng/mL.

References

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12 Gonzales, E., et al., Stability of pain-related medications, metabolites, and illicit substances in urine, *Clinica Chimica Acta*. 416:80-85 (2013)

EasyRA Parameters:

	Qualitative
Primary Wavelength	340
Secondary Wavelength	N/A
Reaction Type	Qual. Kinetic
Reaction Direction	Increase
Calibration Curve	Increase
Reagent Blank	N/A
Sample Blank	N/A
Reaction Time	5.2 Minutes
On-Board Stability	30 Days
Cal Stability	20 Days*

*** with evaporation caps**

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