PPX

cobas®

Propoxyphene Plus

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490959 190	ONLINE DAT Propoxyphene Plus 200 tests	System-ID 07 6920 7	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03304671 190	Preciset DAT Plus I calibrators CAL 1-6 (6 x 5 mL)	Codes 431-436	
03304698 190	C.f.a.s. DAT Qualitative Plus (6 x 5 mL)		
04590856 190	C.f.a.s. DAT Qualitative Plus Clinical (3 x 5 mL)	Code 699	
03312950 190	Control Set DAT I PreciPos DAT Set I (2 x 10 mL) PreciNeg DAT Set I (2 x 10 mL)		
04500873 190	Control Set DAT Clinical PreciPos DAT Clinical (2 x 10 mL) PreciNeg DAT Clinical (2 x 10 mL)		

English

System information

For **cobas c** 311/501 analyzers:

PX3QP: ACN 657: for qualitative assay

PX3SP: ACN 658: for semiquantitative assay

PX3QC: ACN 796: for qualitative assay; using C.f.a.s. DAT Qualitative Plus Clinical

For **cobas c** 502 analyzer:

PX3QP: ACN 8657: for qualitative assay

PX3SP: ACN 8658: for semiquantitative assay

PX3QC: ACN 8796: for qualitative assay; using C.f.a.s. DAT Qualitative Plus Clinical

Intended use

Propoxyphene Plus (PPX) is an in vitro diagnostic test for the qualitative and semiquantitative detection of propoxyphene and its metabolites in human urine on Roche/Hitachi **cobas c** systems at a cutoff concentration of 300 ng/mL. Semiquantitative test results may be obtained that permit laboratories to assess assay performance as part of a quality control program. Measurements obtained by this device are used in the diagnosis of propoxyphene use or abuse and do not measure a level of toxicity. Semiquantitative assays are intended to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas chromatography/mass spectrometry (GC/MS).

Propoxyphene Plus provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. GC/MS is the preferred confirmatory method.[1] Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Summary

Propoxyphene, either as a hydrochloride or napsylate salt, is administrated orally and is used in the treatment of mild to moderate pain disorders.[2,3,4,5] Propoxyphene is structurally similar to methadone, binds to the opioid receptors, and has similar analgesic effects to those seen with morphine-like opioids. When administrated, it is less potent than codeine and when given in combination with aspirin or acetaminophen produces a synergistic effect.[2,3,4,5] Propoxyphene may cause some mild adverse side effects including gastrointestinal pain, vertigo, drowsiness, nausea, constipation, and anorexia. The drug is irritating when administered either intravenously or subcutaneously and abuse by these routes results in damage to veins and soft tissues.[2,5]

Propoxyphene by itself or in conjunction with other drugs, including alcohol, can be toxic and cause fatal results.[5,6] In addition, other toxic effects have been reported such as: pulmonary edema, respiratory depression, cardiotoxicity, hallucinations, and convulsions.[2,4] Once propoxyphene is ingested it is absorbed from the gastrointestinal tract and is metabolized in the liver. The metabolism is extensive with the primary route of metabolism being N-demethylation to form N-norpropoxyphene.[7] Norpropoxyphene is one-fourth to one-half as active an analgesic as propoxyphene but it tends to accumulate in plasma due to a longer half-life.[4] The primary route of release of these metabolites from the human body is through urine and modulations of urinary propoxyphene excretion by urinary pH have been reported.[8]

This assay not only detects propoxyphene, but also has cross-reactivity to the major metabolite norpropoxyphene.[9,10]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS)[9,10] as measured by changes in light transmission. In the absence of sample drug, free antibody binds to drug-microparticle conjugates causing the formation of particle aggregates. As the aggregation reaction proceeds in the absence of sample drug, the absorbance increases.

When a urine sample contains the drug in question, this drug competes with the particle-bound drug derivative for free antibody. Antibody bound to sample drug is no longer available to promote particle aggregation, and subsequent particle lattice formation is inhibited. The presence of sample drug diminishes the increasing absorbance in proportion to the concentration of drug in the sample. Sample drug content is determined relative to the value obtained for a known cutoff concentration of drug.

Reagents - working solutions

R1 Buffer; 0.09 % sodium azide

R2 Propoxyphene antibody (goat polyclonal); buffer; bovine serum albumin; 0.09 % sodium azide

R3 Conjugated propoxyphene derivative microparticles; buffer; 0.09 % sodium azide

R1 is in position B, R2 is in position C, and R3 is in position A.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	8 weeks

Do not freeze.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Urine: Collect urine samples in clean glass or plastic containers. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. Samples should be within the normal physiological pH range of 5-8. No additives or preservatives are required. It is recommended that urine specimens be stored at 2-8 °C and tested within 5 days of collection.[11]

For prolonged storage, freezing of samples is recommended.

Centrifuge highly turbid specimens before testing.

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Adulteration or dilution of the sample can cause erroneous results. If adulteration is suspected, another sample should be collected. Specimen validity testing is required for specimens collected under the *Mandatory Guidelines for Federal Workplace Drug Testing Programs*.[12]

CAUTION: Specimen dilutions should only be used to interpret results of Calc.? and Samp.? alarms, or when estimating concentration in preparation for GC/MS. Dilution results are not intended for patient values. Dilution procedures, when used, should be validated.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 311 **test definition**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 26-49	10 / 26-49
Wavelength (sub/main)	– /505 nm	– /505 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs

Reagent pipetting		Diluent (H_2O)
R1	59 µL	–
R2	59 µL	–
R3	36 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.9 µL	–	–
Decreased	2.9 µL	–	–
Increased	2.9 µL	–	–

cobas c 501/502 **test definition**

	Semiquantitative	Qualitative
Assay type	2-Point End	2-Point End
Reaction time / Assay points	10 / 40-60	10 / 40-60
Wavelength (sub/main)	– /505 nm	– /505 nm
Reaction direction	Increase	Increase
Unit	ng/mL	mAbs

Reagent pipetting		Diluent (H_2O)
R1	59 µL	–
R2	59 µL	–
R3	36 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.9 µL	–	–
Decreased	2.9 µL	–	–
Increased	2.9 µL	–	–

Calibration

Calibrators	*Semiquantitative application* S1-4: Preciset DAT Plus I calibrators, CAL 1-4 0, 150, 300, 600 ng/mL *Qualitative application* S1: C.f.a.s. DAT Qualitative Plus, C.f.a.s. DAT Qualitative Plus Clinical, or Preciset DAT Plus I calibrator - CAL 3 300 ng/mL The drug concentrations of the calibrators have been verified by GC/MS.
Calibration K Factor	For the qualitative application, enter the K Factor as -1000 into the Calibration menu, Status screen, Calibration Result window.
Calibration mode	*Semiquantitative application* Result Calculation Mode (RCM)[a)] *Qualitative application* Linear
Calibration frequency	Full (semiquantitative) or blank (qualitative) calibration - after reagent lot change - as required following quality control procedures

a) See Results section.

Traceability: This method has been standardized against a primary reference method (GC/MS).

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Drug concentrations of the Control Set DAT I and Clinical have been verified by GC/MS.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Results

For the qualitative assay, the cutoff calibrator is used as a reference in distinguishing between preliminary positive and negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Preliminary positive samples are flagged with > Test. Samples producing a negative absorbance value are considered negative. Negative samples are preceded by a minus sign.

The semiquantitation of preliminary positive results should only be used by laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS. It also permits the laboratory to establish quality control procedures and assess control performance.

For the semiquantitative assay, the analyzer computer constructs a calibration curve from absorbance measurements of the standards using a 4 parameter logit-log fitting function (RCM). The logit-log function fits a smooth line through the data points. The analyzer computer uses absorbance measurements of samples to calculate drug or drug metabolite concentration by interpolation of the logit-log fitting function.

NOTE: If a result of Calc.? or Samp.? alarm is obtained, review the Reaction Monitor data for the sample and compare with the Reaction Monitor data for the highest calibrator. The most likely cause is a high concentration of the analyte in the sample, in which case the absorbance value for the sample will be less than that of the highest calibrator. Make an appropriate dilution of the sample using the 0 ng/mL calibrator and rerun the sample. A normal drug-free urine may be substituted for the 0 ng/mL calibrator if the urine and procedure have been validated by the laboratory. To ensure that the sample was not over-diluted, the diluted result, prior to multiplying by the dilution factor, must be at least half the analyte cutoff value. If the diluted result falls below half the analyte cutoff value, repeat the sample with a smaller dilution. A dilution that produces a result closest to the analyte cutoff is the most accurate estimation. To estimate the preliminary positive sample's concentration, multiply the result by the appropriate dilution factor. Dilutions should only be used to interpret results of Calc.? or Samp.? alarms, or when estimating concentration in preparation for GC/MS.

Use caution when reporting results as there are various factors that influence a urine test result, such as fluid intake and other biological factors.

As with any sensitive test for drugs of abuse on automated clinical chemistry analyzers, the possibility exists for analyte carry-over from a sample with an extremely high concentration to a normal (negative) sample which immediately follows it.

Confirm all preliminary positive results by another method.

Limitations - interference[13]

See the "Specific performance data" section of this document for information on substances tested with this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

A preliminary positive result with this assay indicates the presence of propoxyphene and/or its metabolites in urine. It does not measure the level of intoxication.

Interfering substances were added to drug free urine at the concentration listed below. These samples were then spiked to 300 ng/mL using a propoxyphene stock solution. Samples were tested in triplicate (n = 3) on a Roche/Hitachi **cobas c** 501 analyzer. The median % recoveries were calculated and are listed below.

Substance	Concentration Tested	% Propoxyphene Recovery
Acetone	1 %	105
Ascorbic Acid	1.5 %	106
Bilirubin	0.25 mg/mL	100
Creatinine	5 mg/mL	117
Ethanol	1 %	102
Glucose	2 %	111
Hemoglobin	7.5 g/L	86
Human Albumin	0.5 %	119
Oxalic Acid	2 mg/mL	108
Sodium Chloride	0.5 M	117
Sodium Chloride	1 M	127
Urea	6 %	103

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Expected values

Qualitative assay

Results of this assay distinguish preliminary positive (≥ 300 ng/mL) from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

Semiquantitative assay

Results of this assay yield only approximate cumulative concentrations of the drug and its metabolites (see "Analytical specificity" section).

Specific performance data

Representative performance data on the Roche/Hitachi analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined in an internal protocol by running a series of calibrator and controls (repeatability n = 20, intermediate precision n = 100). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Semiquantitative precision

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	227	10	4.5
Level 2	294	10	3.5
Level 3	379	7	1.9

Intermediate precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
Level 1	228	11	4.7
Level 2	298	13	4.2
Level 3	385	10	2.7

Qualitative precision

Cutoff (300)	Number tested	Correct results	Confidence level
0.75x	100	100	> 95 % negative reading
1.25x	100	100	> 95 % positive reading

Lower detection limit of the test

17.9 ng/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the lowest standard (standard 1 + 2 SD, repeatability, n = 21).

Accuracy

100 urine samples, obtained from a clinical laboratory where they screened negative relative to a 300 ng/mL cutoff for propoxyphene in a drug test panel, were evaluated with the Propoxyphene Plus assay. 100 % of these normal urines were negative relative to a 300 ng/mL cutoff. 69 samples obtained from a clinical laboratory, where they screened preliminary positive with a commercially available immunoassay relative to a 300 ng/mL cutoff and were subsequently confirmed by GC/MS, were evaluated with the Propoxyphene Plus assay. 100 % of these samples were positive relative to a 300 ng/mL cutoff. In addition, 10 samples were diluted to a propoxyphene concentration of approximately 75-100 % of the cutoff concentration; and 10 samples were diluted to a propoxyphene concentration of approximately 100-125 % of the cutoff concentration. Data from the accuracy studies described above that fell within the near cutoff value ranges were combined with data generated from the diluted positive urine samples. The following results were obtained with the Propoxyphene Plus assay on the Roche/Hitachi 917 analyzer relative to the GC/MS values.

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Propoxyphene Plus Clinical Correlation (Cutoff = 300 ng/mL)					
		Negative Samples	GC/MS values (ng/mL)[b)]		
			Near Cutoff		431-100260
			221-274	316-382	
Roche/Hitachi 917 analyzer	+	0	0	12	66
	-	100	10	1	0

b) GC/MS values represent the unweighted sum of propoxyphene and norpropoxyphene concentrations.

Additional clinical samples were evaluated with this assay on a Roche/Hitachi **cobas c** 501 analyzer and a Roche/Hitachi 917 analyzer. 100 urine samples, obtained from a clinical laboratory where they screened negative in a drug test panel, were evaluated with the Propoxyphene Plus assay. 100 % of these normal urines were negative relative to the Roche/Hitachi 917 analyzer. 80 urine samples, obtained from a clinical laboratory where they screened preliminary positive with a commercially available immunoassay and were subsequently confirmed by GC/MS, were evaluated with the Propoxyphene Plus assay. 100 % of the samples were positive on both the Roche/Hitachi **cobas c** 501 analyzer and the Roche/Hitachi 917 analyzer.

Propoxyphene Plus Correlation (Cutoff = 300 ng/mL)			
		Roche/Hitachi 917 analyzer	
		+	-
cobas c 501 analyzer	+	80	0
	-	0	100

Analytical specificity

The specificity of this assay for structurally similar compounds was determined by generating inhibition curves for each of the compounds listed and determining the approximate quantity of each compound that is equivalent in assay reactivity to a 300 ng/mL propoxyphene assay cutoff. The following results were obtained on a Roche/Hitachi 917 analyzer.

Compound	**ng/mL Equivalent to 300 ng/mL Propoxyphene**	**Approximate % Cross-reactivity**
Norpropoxyphene	424	71
p-Hydroxypropoxyphene	933	32
Methadone	> 100000	0.1

Drug interference

The following compounds were prepared in aliquots of pooled normal human urine to yield a final concentration of 100000 ng/mL. None of these compounds gave values in the assay that were greater than 0.133 % cross-reactivity.

Acetaminophen
Acetylsalicylic acid
Aminopyrine
Amitriptyline
Amobarbital
d-Amphetamine
l-Amphetamine
Ampicillin
Ascorbic acid
Aspartame
Atropine
Benzocaine
Benzoylecgonine (cocaine metabolite)
Benzphetamine
Brompheniramine
Butabarbital
Caffeine
Calcium hypochlorite
Chlordiazepoxide
Chloroquine
Chlorpheniramine
Chlorpromazine
Clemastine
Cocaine
Codeine
Desipramine
Dextromethorphan
Diazepam
Diphenhydramine
Diphenylhydantoin
Dopamine
Doxepin
Ecgonine
Ecgonine methyl ester
d-Ephedrine
d,l-Ephedrine
l-Ephedrine
Epinephrine
Erythromycin
Estriol
17-Ethynylestradiol
Fenoprofen
Furosemide
Gentisic acid
Glutethimide
Guaiacol glycerol ether
Hydrochlorothiazide
p-Hydroxyamphetamine
Ibuprofen
Imipramine
Isoproterenol
Ketamine
Lidocaine
LSD
MDA
MDMA
Melanin
Meperidine
d-Methamphetamine
l-Methamphetamine
Methapyrilene
Methaqualone
Methylphenidate
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nordiazepam
Norethindrone
l-Norpseudoephedrine
Nortriptyline
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
β-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phenylbutazone
d-Phenylpropanolamine
d,l-Phenylpropanolamine
Phenyltoloxamine
Procaine
Procyclidine
Promethazine
d-Pseudoephedrine
l-Pseudoephedrine
Quinidine
Quinine
Secobarbital
Sulindac
Tetracycline
Δ^9 THC-9-carboxylic acid
Tetrahydrozoline
Thioridazine
Trifluoperazine
d,l-Trihexyphenidyl
Trimipramine
Tripelenamine
Tyramine
Verapamil

References

1 Karch SB, ed. Drug Abuse Handbook. Boca Raton, FL: CRC Press LLC 1998.

2 Hardman JG, Limbird LE, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw Hill Pub Co. 2001.

3 Nickander RC, Emmerson JL, Hynes MD, et al. Pharmacologic and toxic effects in animals of dextropropoxyphene and its major metabolite norpropoxyphene: A Review. Human Toxicol 1984;3:13S-36S.

4 Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 7th ed. Foster City, CA: Biomedical Publications 2004

5 Miller RR. Propoxyphene: A Review. Am J Hosp Pharm 1977;34:413-423.

6 Finkle BS, Caplan YH, Garriott JC, et al. Propoxyphene in postmortem toxicology 1976-1978. J Forensic Sci 1981;26:739-757.

7 Due SL, Sullivan HR, McMahon RE. Propoxyphene: pathways of metabolism in man and laboratory animals. Biomed Mass Spectrum 1976;3:217-226.

8 Karkkainen S, Neuvonen PJ. Effect of oral charcoal and urine pH on dextropropoxyphene pharmacokinetics. Int J Clin Pharmacol Ther Toxicol 1985;23:219-225.

9 McNally AJ, Pilcher I, Wu R, et al. Evaluation of the OnLine Immunoassay for Propoxyphene: Comparison to EMIT II and GC-MS. J. Anal Toxicol 1996;20:537-540.

10 Wu RS, McNally AJ, Pilcher IA, et al. Synthesis of new d-propoxyphene derivatives and the development of a microparticle-based immunoassay for the detection of propoxyphene and norpropoxyphene Bioconjug Chem 1997;8:385-390.

11 Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline. 2nd ed. (C52-A2). Clinical and Laboratory Standards Institute 2007;27:33.

12 Mandatory Guidelines for Federal Workplace Drug Testing Programs. Fed Regist 2010;73:71858-71907.

13 Data on file at Roche Diagnostics.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Significant additions or changes are indicated by a change bar in the margin.

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