

REF 14247-4 4 x 23 mL/9 mL

OXYCODONE ENZYME IMMUNOASSAY (OXY)

Wedges each contain usable volumes of 23 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE

The EasyRA Oxycodone (OXY) reagent is intended for the qualitative determination of oxycodone and its metabolite, oxymorphone, in human urine, at a cutoff value of 100 ng/mL. The assay is designed for prescription use on the EasyRA Clinical Chemistry Analyzer.

The assay provides a rapid screening procedure for determining the presence of oxycodone and its metabolite, oxymorphone, in urine. 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 drug of abuse test result, particularly when the preliminary test result is positive.

SUMMARY AND EXPLANATION OF TEST

Oxycodone is a semi-synthetic narcotic analgesic prescribed for pain management in patients with moderate to severe pain. The drug is approximately equipotent with morphine, but has a higher oral/parenteral dose (3). Similar to morphine, oxycodone can produce drug tolerance and therefore has the potential of being abused. Oxycodone is metabolized by N- and O-demethylation into oxymorphone and noroxycodone. The oxymorphone metabolite is a potent narcotic analgesic and the noroxycodone is relatively inactive. Between 33–61 % of a single dose of oxycodone is excreted in the 24 hour urine as free drug (13–19 %) and conjugated oxycodone (7–29 %), conjugated oxymorphone (13–14 %) and an unknown amount of noroxycodone (3).

Assay Principle

The Oxycodone assay 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 (4). 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, oxycodone-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 oxycodone-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 (R₁): Contains mouse monoclonal anti-oxycodone antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R₂): Contains oxycodone-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

Precautions and Warnings

- *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 (8/16/76).*
- *Do not use the reagents beyond their expiration dates.*

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°C. Opened reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days 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 HANDLING

Urine samples may be collected in plastic or glass containers. Use of plastics such as polyethylene is recommended (5). If the sample cannot be analyzed immediately, it may be refrigerated at 2–8°C for up to three days. For longer storage, keep sample frozen at -20°C and then thaw before use. Studies have shown oxycodone analytes in urine are stable at -20°C for up to six months (6). Samples should be at room temperature (18–25°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 forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

PROCEDURE

Materials Provided

Medica OXY Reagent Wedge, REF 14247 (Qualitative)

Additional Materials Required:

Medica EasyCal Oxycodone Cutoff Calibrator (Oxycodone Cutoff, 100 ng/mL), REF 14680

Medica EasyCal Universal Negative Calibrator (Oxycodone, 0 ng/mL), REF 14799

Medica EasyQC Oxycodone Negative Control (Oxycodone, 75 ng/mL), REF 14791

Medica EasyQC Oxycodone Positive Control (Oxycodone, 125 ng/mL), REF 14796

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

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 and on the EasyRA Clinical Chemistry Analyzer.

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis, use the 100 ng/mL cutoff calibrator. Recalibration should be performed if there is a change in calibrators or reagent lot. Two levels of controls are available for monitoring of each cutoff level. Use the 75 ng/mL and 125 ng/mL controls for the 100 ng/mL cutoff level.

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 Oxycodone Cutoff Calibrator, REF 14680 and Medica EasyCal Universal Negative Calibrator, REF 14799 are required for the calibration of the assay. The calibration interval (30 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

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 Medica Corporation technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

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

Qualitative: The cutoff calibrator, which contains 100 ng/mL of oxycodone, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance ($\Delta\text{mA}/\text{min}$) greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance ($\Delta\text{mA}/\text{min}$) equal to or lower than that obtained with the cutoff calibrator is considered negative.

Procedural Limitations

1. A positive result from the assay indicates only the presence of oxycodone. 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 illegal drugs.
4. Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferences) may influence the urine test results.
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 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 and validated on an EasyRA Clinical Chemistry Analyzer.

Precision:

Qualitative analysis: The calibrator and two levels of controls were evaluated. Typical results ($\Delta\text{mA}/\text{min}$) are as follows:

100 ng/mL Cutoff		Within Run		Total Precision	
N=88 (mA/min)	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	22	22 Neg	88	88 Neg
25 ng/mL	25 %	22	22 Neg	88	88 Neg
50 ng/mL	50 %	22	22 Neg	88	88 Neg
75 ng/mL	75 %	22	22 Neg	88	88 Neg
100 ng/mL	100 %	22	5 Pos/ 17 Neg	88	25 Pos/ 63 Neg
125 ng/mL	125 %	22	22 Pos	88	88 Pos
150 ng/mL	150 %	22	22 Pos	88	88 Pos
175 ng/mL	175 %	22	22 Pos	88	88 Pos
200 ng/mL	200 %	22	22 Pos	88	88 Pos

Accuracy 100 ng/mL Cutoff: Eighty-nine (89) unaltered clinical urine specimens were tested and confirmed with GC/MS or LC/MS. Specimens having an oxycodone concentration greater than 100 ng/mL by GC/MS or LC/MS are defined as positive, and specimens with lower concentrations by GC/MS or LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as $\pm 50\%$ of the cutoff value):

Qualitative Accuracy Study:

100 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreement
Positive	0	0	0	7	38	93.75 %
Negative	20	9	12	3*	0	100.0 %

The following table summarizes the result for the three discordant samples:

Cutoff Value (100 ng/mL)	Sample Testing Method	
	EIA	GC/MS or LC/MS (ng/mL)
Sample #42*	-	108
Sample #43*	-	110
Sample #48*	-	135

Discrepant samples are based on a 100 ng/mL cutoff concentration with 325.7 mAU/min, 314.7 mAU/min, and 325.7 mAU/min absorbance values.

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control, drug-free urine pool spiked with oxycodone was serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

$$y = 0.974x + 1.4518, r^2 = 0.998$$

Target Concentration (ng/mL)	Determined Concentration (ng/mL)	% Recovery
800	801.4	100.2 %
700	657.1	93.9 %
600	585.3	97.6 %
500	494.4	98.9 %
400	383.9	96.0 %
300	302.9	101.0 %
200	190.5	95.2 %
100	102.2	102.2 %
50	50.4	100.8 %
0	1.6	N/A

Specificity

Cross reactivity of various potential interfering drugs were tested by spiking various concentrations of each substance into drug free urine, and then 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 100 ng/mL oxycodone cutoff or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrators.

Structurally Related Compounds: 100 ng/mL Cutoff

Compound	Equivalent to 100 ng/mL (ng/mL)	% Cross Reactivity
Oxycodone	100	110.40%
Hydrocodone	22,300	0.58%
Hydromorphone	14,100	0.68%
Oxymorphone	115	87.91%
Noroxymorphone	1,000	11.96%
Noroxycodone	1,100	9.70%
Codeine	60,000	0.16%
Dextromethorphan	1,000,000	0.01%
Dihydrocodeine	250,000	0.04%
Levorphanol	60,000	0.17%
Naloxone	9,000	0.93%
Norcodeine	1,000,000	0.02%
Morphine	50,000	0.22%
Oxymorphone-glucuronide	85	135.88%
Codeine-6-b-glucuronide	5,000	0.06%
Morphine-3-glucuronide	250,000	0.01%
6-AM	62,100	0.01%
NorBuprenorphine	100,000	0.00%

There is potential that metabolites of the compounds listed above may interfere with the oxycodone assay and cause false results.

Structurally Unrelated Pharmacological Compounds: 100 ng/mL Cutoff

Compound	Target (ng/mL)	% Cross Reactivity	Oxycodone Concentration		
			0 ng/mL	75 ng/mL Control	125 ng/mL Control
Acetaminophen	500,000	0.001%	Neg	Neg	Pos
Acetylsalicylic acid	500,000	0.000%	Neg	Neg	Pos
Amobarbital	500,000	0.001%	Neg	Neg	Pos
Benzoyllecgonine	500,000	0.003%	Neg	Neg	Pos
Bupropion	500,000	0.001%	Neg	Neg	Pos
Caffeine	500,000	0.001%	Neg	Neg	Pos
Chlorpheniramine	500,000	0.001%	Neg	Neg	Pos
Chlorpromazine	500,000	0.001%	Neg	Neg	Pos
d,l-Phenylpropanolamine (Phenethylamine)	250,000	0.003%	Neg	Neg	Pos
d-Ephedrine	500,000	0.003%	Neg	Neg	Pos
l-Ephedrine	300,000	0.001%	Neg	Neg	Pos
d-Methamphetamine	250,000	0.004%	Neg	Neg	Pos
Ecgonine (Ecgonine Methyl-ester)	500,000	0.001%	Neg	Neg	Pos
Meperidine	500,000	0.004%	Neg	Neg	Pos
Methadone	500,000	0.001%	Neg	Neg	Pos
Nicotine	500,000	0.002%	Neg	Neg	Pos
Norpropoxyphene	100,000	0.002%	Neg	Neg	Pos
Phencyclidine	250,000	0.012%	Neg	Neg	Pos
Promethazine	500,000	0.002%	Neg	Neg	Pos
Propranolol	100,000	0.003%	Neg	Neg	Pos
Secobarbital	500,000	0.001%	Neg	Neg	Pos
Trazodone	500,000	0.001%	Neg	Neg	Pos
Tyramine	500,000	0.002%	Neg	Neg	Pos
Valproic Acid	500,000	0.001%	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

Interference: Endogenous Substances: 100 ng/mL Cutoff

The following endogenous compounds were spiked into negative urine and the two levels of controls (75 ng/mL and 125 ng/mL) for the assay. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 100 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked (mg/dL)	Oxycodone Concentration		
		0 ng/mL	75 ng/mL Control	125 ng/mL Control
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	400	Neg	Neg	Pos
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	1500	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Neg	Pos
Sodium Chloride	3000	Neg	Neg	Pos
Oxalic Acid	100	Neg	Neg	Pos
Urea	2000	Neg	Neg	Pos
pH 3	N/A	Neg	Neg	Pos
pH 4	N/A	Neg	Neg	Pos
pH 5	N/A	Neg	Neg	Pos
pH 6	N/A	Neg	Neg	Pos
pH 7	N/A	Neg	Neg	Pos
pH 8	N/A	Neg	Neg	Pos
pH 9	N/A	Neg	Neg	Pos
pH 10	N/A	Neg	Neg	Pos
pH 11	N/A	Neg	Neg	Pos

Specific Gravity: Specific gravity samples ranging in value from 1.000 to 1.0275 were tested with the assay in the presence of 0 ng/mL, 75 ng/mL, 225 ng/mL, and 375 ng/mL of oxycodone and no interference was observed.

Note: All endogenous substances listed above and specific gravity samples, were also tested in qualitative-mode. No interference is observed. The results are identical to the semi-quantitative mode as all samples gave correct positive/negative result corresponding to the cutoff value at 100 ng/mL.

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, **69** (71): 19644 (2004).
3. Baselt, R.C., Advance in Analytical Technology, V 1, Randall C. Baselt edd. (Biomedical Publication, Foster City, CA (572–573).
4. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**:846 (1972).
5. 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).
6. Gonzales, E., et al., Stability of pain-related medications, metabolites, and illicit substances in urine, *Clinica Chimica Acta*, **416**:80–85 (2013).

EasyRA Clinical Chemistry Analyzer

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	2.5 Minutes
On-Board Stability	30 Days
Cal Stability	30 Days*

*with anti-evaporation caps

Performance Characteristics

The results shown below were obtained with Medica EasyRA.

Inaccuracy/Correlation

Eighty-two (82) clinical urine specimens were tested qualitatively with the Oxycodone Enzyme Immunassay (EIA) method on the EasyRA. All results were confirmed with an LC/MS method and are summarized in the table below:

	(0-100 ng/mL) Negative LC/MS	(>100 ng/mL) Positive LC/MS
EasyRA		
Negative (<100 ng/mL)	40	0
Positive (>100 ng/mL)	0	42
% Agreement Negative	100%	
% Agreement Positive	100%	

Imprecision (CLSI, EP5- A2) Qualitative analysis: Precision of oxycodone, samples (Negative, 75 ng/mL and Positive, 125 ng/mL) was performed qualitatively (mAbs/min) on one Medica EasyRA Analyzer. Each of the precision samples was analyzed 20 times per run. The mean, standard deviation and the %CV were calculated for within run precision.

**Within-Run Imprecision (EP5-A2)
Qualitative Results (Cutoff 100 ng/mL)
Qualitative Results (n=20)**

Samples (ng/mL)	Mean (mAbs/Min)	SD (mAbs/Min)	%CV
75	153.4	1.1	0.7%
125	171.9	0.7	0.4%

% Agreement of Qualitative Precision Results with Target Values

Sample	Acceptance Criteria	Results
75 ng/mL	100%	Negative
125 ng/mL	100%	Positive

Qualitative analysis: Two samples of oxycodone were prepared in human urine and analyzed 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. The study followed the protocol defined in EP5-A2. Typical results are as follows:

**Total Imprecision (EP5-A2)
Qualitative Results (Cutoff 100 ng/mL)
Qualitative Results (n=40)**

Mean (ng/mL)	SD	%CV
74.2	5.0	6.7%
125.0	7.4	5.9%

% Agreement of Qualitative Precision Results with Target Values

Sample	Acceptance Criteria	Results
74.2 ng/mL	100%	Negative
124.9 ng/mL	100%	Positive

Manufactured for:



Medica Corporation, 5 Oak Park Drive
Bedford, MA 01730-1413 USA



Emergo Europe, Prinsessegracht 20
2514 AP The Hague, The Netherlands