MEDICA

IVD CE

REF 14243-4 4 x 27 mL/8 mL

OPIATE-QUALITATIVE (OPI)
Wedges each contain usable volumes of 27 mL of R1 reagent and 8 mL of R2 reagent.

INTENDED USE
The EasyRA Opiate (OPI) reagent is intended for the qualitative determination of opiate 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 opiates 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
Opiates are naturally occurring alkaloids derived from the opium poppy. Common opiates include morphine, codeine, and heroin which is a semi-synthetic derivative of morphine[3]. Morphine and codeine are potent analgesics. They are among the most effective treatments of mild to severe pain. These legitimate drugs are often abused for their central nervous system effects. Opiates are absorbed rapidly an metabolized in the liver. Excretion takes place over 2-3 days. The presence of opiates in urine indicates the use of heroin, morphine, codeine or other synthetic opiates structurally related to morphine[4,5,6].

PRINCIPLE OF THE PROCEDURE
The opiate 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, opiate-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 opiate-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-morphine antibody, glucose-6-phosphate (G6P), Nicotinamide adenine dinucleotide (NAD), stabilizers and sodium azide as preservative.

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

Precautions
1. This test is for in-vitro diagnostic use only. Harmful if swallowed.
2. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
3. 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).
4. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
5. 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° C. The 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 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[8]. 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[9]. For longer storage keep sample frozen at -20°C and then thaw before use. Studies have shown that opiate analytes in urine are stable at -20°C up to 12 months[10]. Samples should be brought to a room temperature of 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 both samples should be forwarded to the laboratory for testing. Handle all urine specimens as if they are potentially infectious.

PROCEDURE

Materials Provided:
Medica OPI Reagent Wedge, REF 14243 (Qualitative)

Additional materials required:
Medica EasyCal Opiate Cutoff Calibrator (Opiates Cutoff, 300 ng/mL), REF 14776
Medica EasyQC Opiate Negative Control (Opiates, 225 ng/mL), REF 14790
Medica EasyQC Opiate Positive Control (Opiates, 375 ng/mL), REF 14795
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 Opiate Cutoff Calibrator, REF 14776 is required for the calibration of the assay. The calibration interval (1day 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 morphine is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (mA/min) equal to, or greater than, that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance value 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 opiates.
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 thirty-four (134) 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:

EasyRA	(<150ng/mL) Negative LC/MS	Near Cutoff (150-300ng/mL) Negative LC/MS	Near Cutoff (300-450ng/mL) Positive LC/MS	(>450ng/ml) Positive LC/MS
Positive(>300ng/mL)	0	0	10	47
Negative(<300ng/mL)	62	12	0	3

% Agreement	100.00%
% Agreement	95.00%

*LC/MS data represents the total of morphine plus cross reactive species.

Imprecision(CLSI, EP5-A2)
Qualitative analysis: Nine samples of opiates 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 measured for each reading. The study followed the protocol defined in EP5-A2 (CLSI). 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	120.0	1.2	0.85%
75	151.6	0.8	0.50%
150	173.6	0.9	0.49%
225	186.8	0.8	0.41%
300	195.9	0.8	0.43%
375	202.9	1.0	0.50%
450	210.1	1.1	0.51%
525	215.3	1.1	0.49%
600	220.7	1.1	0.51%

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

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	120.0	1.2	1.02%
75	151.6	1.1	0.71%
150	173.6	1.4	0.83%
225	186.8	1.6	0.87%
300	195.9	1.4	0.70%
375	202.9	1.4	0.71%
450	210.1	1.3	0.64%
525	215.3	1.8	0.83%
600	220.7	1.7	0.77%

% 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	3	80	100%
300	80	0	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 on the Hitachii 717. Test compounds were spiked into the drug-free urine calibrator matrix 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 concentration of the compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Cross-Reactant	Concentration (ng/mL)	Cross-Reactivity
Morphine	300	Positive
Codeine	150	Positive
Dihydrocodeine	400	Positive
Hydrocodone	300	Positive
Hydromorphone	600	Positive
Levorphanol	600	Positive
Morphine-3-glucuronide (in morphine equiv.)	625	Positive
Morphine-6-glucuronide	550	Positive
Norcodeine	7000	Positive
Oxycodone	2000	Positive
Oxymorphone	6000	Positive
Thebaine	400	Positive
	Concentration (µg/mL)	
Acetylsalicylic Acid	3000	Negative
Albuterol	3000	Negative
Amitriptyline	50	Negative
Amobarbital	3000	Negative
d-Amphetamine	3000	Negative
Benzoylecgonine	3000	Negative
Bupropion	1000	Negative
Caffeine	3000	Negative
Chlorpromazine	80	Negative
Clomipramine	30	Negative
Cycloazocine	300	Negative
Desipramine	130	Negative
Dextromethorphan	40	Negative
Doxepin	175	Negative
Ecqonine	3000	Negative
Ephedrine	1400	Negative
Fentanyl	3000	Negative
Fluoxetine	800	Negative
Fluphenazine	3000	Negative
Imipramine	20	Negative
Lidocaine	3000	Negative
Maprotiline	600	Negative
Meperidine	25	Negative
Methadone	1000	Negative
Methapyrilene	300	Negative
Methaqualone	3000	Negative
Metronidazole	700	Negative
Nalbuphine	3000	Negative
Naloxone	85	Negative
Naltrexone	3000	Negative
Nicotine	800	Negative
Normorphine	30	Negative
Nortriptyline	110	Negative
Oxazepam	3000	Negative
Phencyclidine	900	Negative
Phenobarbital	3000	Negative
Propoxyphene	260	Negative
Secobarbital	3000	Negative
Thioridazine	70	Negative
Tramadol	1000	Negative
Valproic Acid	3000	Negative

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

REFERENCES

1. Urine Testing for Drugs 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, vol. 53, No. 69, ppl 11970 (1988).
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4. Glare P.A., and T.D. Walsh. Clinical Pharmacokinetics of morphine. *Ther. Drug Monit*. 13: 1 (1991).
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6. Hasselstrom, J. and J. Sawe. Morphine pharmacokinetics and metabolism in humans: Enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. *Clin Pharmacokinet*. 24: 344 (1993).
7. Rubenstein, K.E., R.S. Schneider, and E.F. Ullman, Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, 47, 846 (1972).
8. 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).
9. Gonzales, E., et al., Stability of pain-related medications, metabolites, and illicit substances in urine, *Clinica Chimica Acta.* 416:80-85 (2013)
10. Chang, B.L., Huang, M.K., and Tsai, Y.Y., Total morphine stability in urine specimens stored under different conditions, *J. Anal. Toxicol.,* 24(6):442-447 (2000).

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	2.4 Minutes
On-Board Stability	30 Days
Cal Stability	1 Day*

*with evaporation caps

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