MEDICA

IVD CE

REF 14241-4 4 x 23 mL/9 mL

COCAINE-QUALITATIVE IMMUNOASSAY (COC)
Wedges each contain usable volumes of 23 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE

The EasyRA Cocaine (COC) reagent is intended for the qualitative determination of cocaine 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 cocaine in urine. The assay provides only a preliminary qualitative 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 method.[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

Cocaine (methylbenzoylecgonine) is an alkaloid found in the plant *Erythroxylon coca*, which is principally grown in South America. Cocaine is a central nervous system stimulant; however, it also exhibits numerous undesirable side effects including cardiac toxicity and behavior response such as paranoia and hallucinations[3]. Cocaine is rapidly metabolized with less than 5% excreted unchanged in urine. The major metabolite is benzoylecgonine. Cocaine metabolites are detected in urine for 1-3 days after moderate use.[4,5] However for long term heavy use, the metabolites may be found in urine for up to 3 weeks.[6,7]

PRINCIPLE OF THE PROCEDURE

The cocaine assay is a homogeneous enzyme immunoassay which provides qualitative results relative to a single calibration cutoff value[8]. 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, benzoylecgonine-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 benzoylecgonine-labeled G6PDH then exhibits its maximal enzyme activity.

Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in and absorbance increase that can be measured spectrophotometrically at 340nm.

REAGENTS

Antibody/Substrate Reagent (R1): Contains monoclonal anti-benzoylecgonine antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers in buffer.

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

Precautions and Warnings

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 ^{o}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 adsorb drugs. Use fresh urine specimen for the test. If the sample cannot be analyzed immediately, it may be stored refrigerated for up to 3 days. For longer storage keep sample frozen and then thaw before use. Samples should be to room temperature of 18-25^{o}C for testing. Samples with high turbidity should be centrifuged before analysis. Fresh and properly stored urine samples generally are within this range. Sample with pH out of the range should be adjusted to be within this range with 1N HCl or 1N NaOH before testing.

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 COC Reagent Wedge, REF 14241 (Qualitative)

Additional materials required:
Medica EasyCal Cocaine Cutoff Calibrator (Cocaine Cutoff, 300 ng/mL), REF 14654
Medica EasyQC Cocaine Negative Control (Cocaine, 225 ng/mL), REF 14763
Medica EasyQC Cocaine Positive Control (Cocaine, 375 ng/mL), REF 14768
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 Cocaine Cutoff Calibrator, REF 14654 is required for the calibration of the assay. The calibration interval (26 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 benzoylecgonine is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit time (mA/min) 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 cocaine.
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 twenty-six (122) 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	0	7	51
Negative (<300 ng/ml)	51	11	2	0
% Agreement	100%			
% Agreement	96.60%			

*LC/MS data represents the total benzoylecgonine.

Imprecision/Correlation (CLSI-EPSA2)

Qualitative Analysis: Nine samples of Cocaine (COC) spread evenly throughout the range of 0-600 ng/mL were prepared 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. 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	61.0	0.8	1.29%
75	65.3	0.6	0.89%
150	73.1	0.8	1.11%
225	86.5	1.2	1.38%
300	104.2	0.9	0.91%
375	119.7	1.1	0.90%
450	129.0	1.0	0.80%
525	135.7	1.0	0.76%
600	142.3	1.1	0.75%

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

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	61.0	0.8	1.35%
75	65.3	0.7	1.06%
150	73.1	1.0	1.44%
225	86.5	1.7	1.99%
300	104.2	1.9	1.84%
375	119.7	1.6	1.37%
460	129.0	1.4	1.09%
525	135.7	1.9	1.38%
600	142.3	1.3	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	3	77	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).

COMPOUND	CONCENTRATION (NG/ML)	CROSS-REACTIVITY
Benzoylecgonine	0.3	Positive
Cocaine	30	Positive
Norcocaine	60	Positive
Ecgonine, Methyl Ester	350	Positive
	µg/mL	
Acetaminophen	1500	Negative
Acetylsalicyclic Acid	1500	Negative
Amobarbital	1000	Negative
Amphetamine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Codeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1500	Negative
Methadone	1000	Negative
Morphine	2000	Negative
Nicotine	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	1000	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

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).
3. Goodman, L.S. and A. Gilman. The Pharmacological Basis of Therapeutics, 4th Edition, 380, The MacMillan Co,, 1970.
4. Bouknight, L.G. and R.R. Bouknight. Cocaine- A Particularly Addictive Drug, *Postgrad. Med*. 83, 115, 1988.
5. Benowitz, N.L. Clinical Pharmacology and Toxicology of Cocaine, *Pharmacol. & Toxicol.* 72, 3, 1993.
6. Weiss, R.D. and F.H. Gawin. Protracted Elimination of Cocaine Metabolites in Long-term, High-dose Cocaine Abusers, *Am. J. Med.,* 85, 879, 1988.
7. Burke, W.M. et al., Prolong Presence of Metabolite in Urine after Compulsive Cocaine Use, *J. Clin. Psychiatry*, 51, 145, 1990.
8 Rubenstein, K.E., R.S. Schneider, and E.F. Ullman, Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun,* 47, 846 (1972).

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	26 Days*

*with evaporation caps

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