MEDICA

IVD CE

REF 14252-4 4 x 24 mL/9 mL

ECSTASY- QUALITATIVE (XTC)
Wedges each contain usable volumes of 24 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE
The EasyRA Ecstasy (XTC) reagent is intended for the qualitative determination of ecstacy in human urine at a cutoff value of 500 ng/mL. For *in-vitro* diagnostic use only.

The assay provides a rapid screening procedure for determining the presence of ecstasy 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 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
Ecstasy drugs are a group of amphetamine derivatives, including MDMA (3,4-methylenedioxymethamphetamine), MDA (3,4-methylenedixoyamphetamine), and MDEA (3,4-methylenedioxyethylamphetamine). They are central nervous system (CNS) stimulants. At light dose, ecstasy drugs produce euphoria, increase self-awareness. However, they are popularly abused for their psychotropic effects at high dose and become hallucinogenic and loss control of behavior. Toxic overdose causes depression, uncontrolled body fluid excretion, cardiac arrhythmias, and sleep disorder. Since there is no known medical application of ecstasy drugs with high abuse potential, US DEA list both MDMA and MDA as schedule I drugs. After ingestion of the drug, MDMA is known to metabolize to MDA by demethylation. Within human body, most of the drug is eliminated through urinary excretion. Most of the urinary excretion is unchanged MDMA with small fraction of MDA. Other urinary excretion include mono- and dihydroxy derivatives appear as glucuronide conjugates[3] . Detection of MDMA or its metabolites in urine indicates use of ecstasy.

PRINCIPLE OF THE PROCEDURE
The ecstasy assay is a homogeneous enzyme immunoassay[4]which provides qualitative results relative to a single calibration cutoff value. The assay is based on competition between ecstasy in the sample and ecstasy labeled with the enzyme glucose-6-phosphate dehydrogenase (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, ecstasy-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 would bind to free drug, the unbound ecstasy-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 340 nm.

REAGENTS
Antibody/Substrate Reagent (R1): Contains monoclonal anti-ecstasy antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R2): Contains ecstasy-labeled glucose-6-phosphate dehydrogenase (G6PDH), buffer and sodium azide (0.09 %) as a 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 ^{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 absorb drugs. Use of plastics such as polyethylene is recommended[5]. 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[6]. For longer storage keep sample frozen at -20°C and thaw then before use. Studies have shown that MDMA analytes in urine are stable at -20°C up to 17 months[7]. 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 XTC Reagent Wedge, REF 14252 (Qualitative)

Additional materials required:
Medica EasyCal Ecstasy Cutoff Calibrator (Ecstasy Cutoff, 500 ng/mL), REF 14700
Medica EasyQC Ecstasy Negative Control (Ecstasy, 375 ng/mL), REF 14764
Medica EasyQC Ecstasy Positive Control (Ecstasy, 625 ng/mL), REF 14769
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or
Medica Cleaner Wedge – Chemistry, REF 10661
Medica 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 C ap is required to guarantee on-board calibration stability.

Calibration
Medica EasyCal Ecstasy cutoff Calibrator, REF 14700 is required for the calibration of the qualitative assay. The calibration interval (10 days maximum) with evaporation caps is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change tin 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 500 ng/mL of ecstasy, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit times (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 ecstasy.
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 seven (107) 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	(<250ng/mL) Negative LC/MS	Near Cutoff (250-500ng/mL) Negative LC/MS	Near Cutoff (500-750ng/mL) Positive LC/MS	(>750ng/ml) Positive LC/MS
Positive(>500ng/mL)	1	1	11	43
Negative(<500ng/mL)	40	11	0	0

% Agreement Negative	96.20%
% Agreement Positive	100.00%

Imprecision (CLSI-A2)

Qualitative analysis: Nine samples of Ecstasy spread evenly throughout the range of 0-1000 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	93.1	0.34	0.37
125	101.5	0.33	0.33
250	117.7	0.38	0.38
375	146.2	0.63	0.43
500	164.5	0.70	0.43
625	176.6	0.74	0.42
750	184.3	0.70	0.38
875	189.1	0.80	0.43
1000	192.7	0.83	0.43

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

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	93.1	0.72	0.77
125	101.5	0.81	0.79
250	117.7	0.83	0.70
375	146.2	0.94	0.64
500	164.5	1.06	0.65
625	176.6	1.11	0.63
750	184.3	1.06	0.58
875	189.1	1.18	0.62
1000	192.7	1.11	0.58

% Agreement of Qualitative Precision Results with Target Values

Samples (ng/mL)	Number Positive	Number Negative	% Agreement
0	0	80	100%
125	0	80	100%
250	0	80	100%
375	0	80	100%
500	28	52	N/A
625	80	0	100%
750	80	0	100%
875	80	0	100%
1000	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 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
MDMA	500	Positive
MDEA	500	Positive
MDA	1,000	Positive
d,*l*-BDB*	1,200	Positive
PMMA*	1,400	Positive
MBDB*	2,000	Positive
PMA*	4,000	Positive
HMMA*	100,000	Negative

	µg/mL	
Acetaminophen	1000	Negative
Acetylsalicylic acid	1000	Negative
Amitriptyline	1000	Negative
Amobarbital	1000	Negative
d-Amphetamine	1000	Negative
l-Amphetamine	100	Negative
Benzoylecgonine	1000	Negative
Bupropion	1000	Negative
Caffeine	1000	Negative
Chlorpheniramine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine	1000	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
Ecgonine	1000	Negative
Ephedrine	1000	Negative
Imipramine	1000	Negative
Lidocaine	1000	Negative
Meperidine	1000	Negative
Methadone	1000	Negative
d-Methamphetamine	800	Negative
l-Methamphetamine	50	Negative
Methaqualone	1000	Negative
Morphine	1000	Negative
Nortriptyline	1000	Negative
Oxazepam	1000	Negative
Phencyclidine	1000	Negative
Phenobarbital	1000	Negative
Phentermine	300	Negative
d,*l*-Phenylpropanolamine	1000	Negative
Promethazine	1000	Negative
Propoxyphene	1000	Negative
Ranitidine	1000	Negative
Secobarbital	1000	Negative
Valproic Acid	1000	Negative

***d,l- BDB**, 3,4-Methylenedioxyphenyl-2-butanamine; **PMMA**, *p*-Methoxymethamphetamine; **PMA**, *p*-Methoxyamphetamine; **MBDB**, N-Methyl-1-(3,4- methylenedioxyphenyl)-2-butanamine; **HMMA**, 4-Hydroxy-3-methoxymethamphetamine

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 Baselt RC and Cravey, RH, "Disposition of Toxic Drugs and Chemicals in Man", Chemical Toxicology Institute, 4th ed. Foster City, CA., 1995
4 Rubenstein, K.E., R.S. Schneider, and E.F. Ullman, 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)
7 Jimenez, C., de la Torre, R., Ventura, M., Segura, J. and Ventura, R., Stability studies of amphetamine and ephedrine derivatives in urine, *Journal of Chromatography B*, 843: 84-93 (2006).

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	2.8 Minutes
On-Board Stability	30 Days
Cal Stability	10 Days*

*With evaporation caps

Manufactured for:

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

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