MEDICA

IVD CE

REF 14240-4 4 x 27mL/ 8mL

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

INTENDED USE
The EasyRA Amphetamines (AMP) reagent is intended for the qualitative determination of amphetamines in human urine at a cutoff value of 1000 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 amphetamines 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
Amphetamines are a class of phenethylamine drugs that imitate the stimulating actions of the endogenous neurotransmitter[3]. The ability of amphetamines to alleviate fatigue, improve mental and physical performances, elevate mood, and produce euphoria has led to the abuse of these legitimate drugs[4]. Amphetamines are psychologically and physiologically addicting. Due to its ease of manufacture, methamphetamine is the most abused amphetamine[5,6]. Amphetamines are rapidly adsorbed from the gastrointestinal tract and either metabolized in the liver or excreted in urine unchanged[3,4]. Amphetamines may be detected in urine 3-4 days after administration.

PRINCIPLE OF THE PROCEDURE
The amphetamines 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, amphetamine -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 amphetamine -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 2 monoclonal anti-amphetamine antibodies, glucose-6-phosphate (G6P), Nicotinamide adenine dinucleotide (NAD), stabilizers and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R2): Contains amphetamine-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a 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°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. For longer storage keep sample frozen at -20°C and then thaw before use. Studies have shown that amphetamine analytes in urine are stable at -20°C for up to 17 months9. 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.

Materials Provided:
Medica AMP Reagent Wedge, REF 14240 (Qualitative)
Additional materials required:
Medica EasyCal Amphetamines Cutoff Calibrator (Amphetamines Cutoff, 1000 ng/mL), REF 14650
Medica EasyQC Amphetamines Negative Control (Amphetamines, 750 ng/mL), REF 14761
Medica EasyQC Amphetamines Positive Control (Amphetamines,1250 ng/mL), REF 14766
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 Amphetamine (AMP) Calibrator, REF 14650 is required for the calibration of the qualitative assay. The calibration interval (11 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 1000 ng/mL of methamphetamine, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit time (mA/min) 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. A positive result from the assay indicates only the presence of amphetamines. 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 amphetamines.
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.
6. 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 fifty-one (151) 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:

	(<750ng/mL) LC/MS	Near Cutoff (750-1000ng/mL) LC/MS	Near Cutoff (1000-1250ng/mL) LC/MS	(>1250ng/ml) LC/MS
EasyRA	**Negative**	**Negative**	**Positive**	**Positive**
Positive(>1000ng/mL)	3	1	14	70
Negative(<1000ng/mL)	52	11	0	0
% Agreement Negative		94%		
% Agreement Positive		100%		

***** LC/MS data represents the total of amphetamine plus methamphetamine

Imprecision (CLSI, EP5-A2)

Qualitative analysis: Nine samples of Amphetamine spread evenly throughout the range of 0-2000 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	121.8	0.5	0.44%
250	134.2	0.7	0.52%
500	143.3	0.6	0.45%
750	157.2	0.9	0.57%
1000	173.8	0.9	0.50%
1250	180.4	0.9	0.49%
1500	186.4	1.0	0.54%
1750	191.5	0.8	0.40%
2000	197.2	0.8	0.39%

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

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	121.8	0.8	0.64%
250	134.2	1.0	0.75%
500	143.3	1.1	0.76%
750	157.2	1.1	0.69%
1000	173.8	1.1	0.64%
1250	180.4	1.2	0.66%
1500	186.4	1.3	0.70%
1750	191.5	1.2	0.61%
2000	197.2	1.3	0.64%

% Agreement of Qualitative Precision Results with Target Values

Samples (ng/mL)	Number Positive	Number Negative	% Agreement
0	0	80	100%
250	0	80	100%
500	0	80	100%
750	0	80	100%
1000	80	0	N/A
1250	80	0	100%
1500	80	0	100%
1750	80	0	100%
2000	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
d-Amphetamine	1000	Positive
d-Methamphetamine	1000	Positive
MDA	2800	Positive
MDMA	2500	Positive

	Concentration (µg/mL)	
Acetaminophen	3000	Negative
Acetylsalicylic acid	3000	Negative
Amorbarbital	3000	Negative
l-Amphetamine	24	Negative
Benzoylecgonine	3000	Negative
Benzphetamine	2000	Negative
Bromopheniramine	3000	Negative
Bupropion	2000	Negative
Buspirone	3000	Negative
Caffeine	3000	Negative
Chlorpheiramine	3000	Negative
Chlorpromazine	3000	Negative
Codeine	3000	Negative
Dextromethorphan	3000	Negative
d-Ephedrine	3000	Negative
d, l-Ephedrine	700	Negative
l-Ephedrine	400	Negative
Fenfluramine	7	Negative
3-Hydroxy-Tyramine	1700	Negative
Isoxsuprine	3000	Negative
l-Methamphetamine	10	Negative
Meperidine	3000	Negative
Mephentermine	50	Negative
Methadone	3000	Negative
Methapyrilene	3000	Negative
Methaqualone	3000	Negative
Morphine	3000	Negative
Oxazepam	3000	Negative
Phencyclidine	1000	Negative
Phendimetrazine	300	Negative
Phenethylamine	40	Negative
Phenmetrazine	75	Negative
Phenobarbital	3000	Negative
Phenothiazine	100	Negative
Phentermine	40	Negative
Phenylephrine	500	Negative
d-Phenylpropanolamine	2500	Negative
d, l-Phenylpropanolamine	500	Negative
l-Phenylpropanolamine	240	Negative
Procainamide	800	Negative
Promethazine	3000	Negative
Propoxyphene	3000	Negative
Propranolol	3000	Negative
d-Pseudoephedrine	250	Negative
l-Pseudoephedrine	2500	Negative
Ranitidine	800	Negative
Scopolamine	3000	Negative
Secobarbital	3000	Negative
Sertraline	1000	Negative
Thioridazine	3000	Negative
Trazodone	2900	Negative
Trifluoperazine	3000	Negative
Triflupromazine	3000	Negative
Triprolidine	3000	Negative
Tyramine	600	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.
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5. Cox, T.C., et al, Drugs and Drug Abuse, Addiction Research Foundation, pp. 153-156, 1983.
6. Leshner, A.L. Club Drugs, Community Drug Alert Bulletin, www.clubdrugs.org. NIDA's Community Epidermiology Work group. 2001.
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. 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	2.4 Minutes
On-Board Stability	30 Days
Cal Stability	11 Days*

*with evaporation caps

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