

REF 14246-4

4 x 24 mL/9 mL

BARBITURATE QUALITATIVE (BAR)

Wedges each contain usable volumes of 24 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE

The EasyRA Barbiturate (BAR) reagent is intended for the qualitative measurement of barbiturates in human urine at a cutoff value of 200 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 barbiturates 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

Barbiturates are nervous system depressants, and usually taken orally, injected intravenously or intramuscularly. They are absorbed rapidly.³ Barbiturates are classified based on their duration of action, ranging from few minutes to a day or more. Barbiturate abuse can lead to respiratory depression or coma in the severe case. Most commonly abused barbiturates are the short acting ones, including pentobarbital and secobarbital. Frequently abused of long acting barbiturate is phenobarbital which excreted in urine primarily appear unchanged.^{4,5} Detection of barbiturates or their metabolites in urine can be used as an indication for use of barbiturates. The barbiturate enzyme immunoassay is designed to test for barbiturate and barbiturate metabolites in human urine.

PRINCIPLE OF THE PROCEDURE

The barbiturate assay is a homogeneous enzyme immunoassay 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 dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent.⁶ 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, barbiturate-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 would bind to free drug, the unbound barbiturate-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 a mixture of monoclonal and polyclonal anti-barbiturates antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R2): Contains barbiturates-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 recommended⁷. 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 thaw then before use. Studies have shown that barbiturate analytes in urine are stable at -20°C for up to 14 days^{8,9}. 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 BAR Reagent Wedge, REF 14246 (Qualitative)

Additional materials required:

Medica EasyCal Barbiturate Cutoff Calibrator (Barbiturate Cutoff, 200 ng/mL), REF 14676

Medica EasyQC Barbiturate Negative Control (Barbiturate, 100 ng/mL), REF 14782

Medica EasyQC Barbiturate Positive Control (Barbiturate, 300 ng/mL), REF 14783

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 Barbiturate Cutoff Calibrator, REF 14676 is required for the calibration of the qualitative assay. The calibration interval (7 day 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 200 ng/mL of secobarbital, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance per unit time (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. A positive result from the assay indicates only the presence of barbiturates. 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 barbiturates.
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-three (153) 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:

	(<100ng/mL) Negative LC/MS	Near Cutoff (100-200ng/mL) Negative LC/MS	Near Cutoff (200-3000ng/mL) Positive LC/MS	(>3000ng/mL) Positive LC/MS
EasyRA Positive(>200ng/mL)	0	0	9	60
EasyRA Negative(<200ng/mL)	70	11	3	0
% Agreement Negative		100.00%		
% Agreement Positive		95.80%		

Imprecision (CLSI, EP5-A2)

Qualitative analysis: Nine samples of Barbiturates (as secobarbital) spread evenly throughout the range of 0-400 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 (Cut-off 200ng/mL)

Qualitative Results (n=80)

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	197.3	1.02	0.52
50	205.0	0.76	0.37
100	214.1	0.84	0.39
150	224.8	1.04	0.46
200	235.3	0.85	0.36
250	243.3	0.91	0.38
300	250.7	1.22	0.49
350	256.4	0.81	0.32
400	260.5	1.15	0.44

Total Imprecision (EP5-A2)

Qualitative Results (Cut-off 200ng/mL)

Qualitative Results (n=80)

Samples (ng/ml)	Mean (mA/Min)	SD (mA/Min)	%CV
0	197.3	1.46	0.74
50	205.0	1.29	0.63
100	214.1	1.56	0.73
150	224.8	1.57	0.70
200	235.3	1.41	0.60
250	243.3	1.47	0.60
300	250.7	2.13	0.85
350	256.4	1.75	0.68
400	260.5	2.11	0.81

% Agreement of Qualitative Precision Results with Target Values

Samples (ng/mL)	Number Positive	Number Negative	% Agreement
0	0	80	100%
50	0	80	100%
100	0	80	100%
150	0	80	100%
200	1	79	N/A
250	80	0	100%
300	80	0	100%
350	80	0	100%
400	80	0	100%

Specificity

Various potentially interfering substances were tested for cross-reactivity with the assay on the Hitachi 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 non-barbiturate compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Barbiturate Compounds	Concentration (ng/mL) at 200 ng/mL	Cross-Reactivity
Secobarbital	200	Positive
Allobarbital	1000	Positive
Amobarbital	2000	Positive
Aprobarbital	450	Positive
Barbital	7000	Positive
Butabarbital	800	Positive
Butalbital	470	Positive
Cyclopentobarbital	250	Positive
Pentobarbital	650	Positive
Phenobarbital	400	Positive
Thiopental	1300	Positive
Non-barbiturates		
	µg/mL	
Acetaminophen	1000	Negative
Acetylsalicylic acid	1000	Negative
Amitriptyline	1000	Negative
Amphetamine	1000	Negative
Benzoyllecgonine	1000	Negative
Caffeine	1000	Negative
Chlorpromazine	1000	Negative
Cocaine	1000	Negative
Codeine	1000	Negative
Dextromethorphan	1000	Negative
Ephedrine	1000	Negative
Imipramine	1000	Negative
Meperidine	1000	Negative
Methadone	1000	Negative
Methamphetamine	1000	Negative
Methaqualone	1000	Negative
Morphine	1000	Negative
Nortriptyline	1000	Negative
Promethazine	1000	Negative
Propoxyphene	1000	Negative
Valproic Acid	1000	Negative
Lidocaine	1000	Negative
Chlorpheniramine	1000	Negative
Ecgonine	1000	Negative
Bupropion	1000	Negative
Ranitidine	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 Hoffmann FE. A Handbook on Drug and Alcohol Abuse; The biomedical aspects, New York, NY; Oxford University Press, 1983.
- 4 Ellenhorn MJ, Braceloux DG, Medical Toxicology, New York, NY; Elsevier Science Publishing Company, Inc.; 1988, 575-580.
- 5 Wyngaarden JB, Smith LH Jr., eds. Cecil Textbook of Medicine, Philadelphia, PA: WB Saunders Co.; 1988, 53-54.
- 6 Rubenstein, K.E., R.S. Schneider, and E.F. Ullman, Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, Biochem Biophys Res Commun, 47, 846 (1972).
- 7 Yahya, A.M., McElney, J.C., and D'Arcy, P.F. Drug absorption to glass and plastics, *Drug Metabol Drug Interact*, 6(1):1-45 (1988)
- 8 Rockerbie, R.A., and Campbell, D.J. Effect of Specimen Storage and Preservation on Toxicological Analyses of Urine, *Clin. Biochem*, 11(3): 77-81 (1978).
- 9 Queen, C.A., Stability of certain drugs of abuse in urine specimens, *Clinical Chemistry*, 18(11):1442 (1972).

EasyRA Parameters:

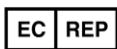
	Qualitative
Primary Wavelength	340
Secondary Wavelength	N/A
Reaction Type	Qual. Kinetic
Reaction Direction	Increase
Calibration Curve	Increase
Reagent Blank	N/A
Sample Blank	N/A
Reaction Time	2.8 Minutes
On-Board Stability	30 Days
Cal Stability	7 Days*

*With evaporation caps

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