DRG One Step Barbiturates (RAP-3009)

INTENDED USE

The One Step Barbiturates Screening Test is a rapid, qualitative immunoassay for the detection of Barbiturate compounds in urine. This assay is calibrated to react with Secobarbital at a cutoff concentration of 200 ng/mL. Other Barbiturates also react (see the "Cross-Reacting Substances" section of this test instruction sheet.) This assay is intended for professional use. This test provides only a preliminary test result. A more specific alternate testing method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method. Other confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, certainly when preliminary positive results are observed.

SUMMARY & EXPLANATION OF THE TEST

Barbiturates form a large class of abused pharmaceuticals. These products are anxiolytic, sedative/hypnotic, anticonvulsant and anesthetic drugs. As CNS depressants, barbiturates exert effects on excitatory and inhibitory synaptic neurotransmission. Ultra short-acting barbiturates used for anesthesia, such as Pentobarbital, depress excitatory neuronal transmission to a greater extent than anti-convulsant barbiturates such as Phenobarbital. Barbiturates are rapidly and completely absorbed with nearly 100% bioavailability. Short-acting barbiturates are primarily excreted in urine as metabolites, while long-acting barbiturates are primarily excreted unchanged. Ratios of drugs to metabolites excreted vary, dependent upon duration of action. Urine based screening tests for drugs of abuse range from complex analytical procedures to simple immunoassay tests. The sensitivity and rapidity of the immunoassays have made them the most accepted method of preliminary screening for drugs of abuse in urine. This allows the laboratory to eliminate the large number of negative specimens and focus on the smaller number of initially positive samples.

PRINCIPLES OF THE PROCEDURE

The One Step Barbiturates Screening Test is a competitive immunoassay that is used to screen for the presence of Barbiturates in urine. It is a chromatographic absorbent device in which drugs or drug metabolites in a sample compete with drug / protein conjugate immobilized on a porous membrane for a limited number of antibody /dye conjugate binding sites. The test device employs a unique combination of monoclonal and polyclonal antibodies to selectively identify Barbiturates in urine with a high degree of confidence. In the procedure, urine is added to the test device's "SAMPLE" well with the aid of a plastic transfer pipette. The urine is absorbed into the device by capillary action, mixes with the antibody / dye conjugate, and flows across the pre-coated membrane.

When sample Barbiturate levels are below 200 ng/mL (the detection sensitivity of the test), antibody / dye conjugate binds to the drug / protein conjugate immobilized in the Test Region (**T**) of the device. This produces a colored Test Band that, regardless of its intensity, indicates a negative result.

When sample Barbiturate levels are at or above 200 ng/mL, the free drug in the sample binds to the antibody/dye conjugate, preventing the antibody / dye conjugate from binding to the drug / protein conjugate immobilized in the Test Region (**T**) of the device. This prevents the development of a distinct colored band, indicating a potentially positive sample. In either case, a colored Control Band is produced in the Control Region (**C**) by a non-specific antibody / dye conjugate reaction. This band serves as a built-in quality control device that demonstrates antibody recognition and reactivity as well as confirmation that the test result is valid.

REAGENTS & MATERIALS SUPPLIED

1. 25 test devices containing:
   a. Monoclonal anti-Secobarbital antibody / colloidal gold conjugate in a protein matrix containing 0.1% sodium azide coated in the sample path
   b. Secobarbital derivative / protein conjugate immobilized as a line in the test region
   c. Goat anti-mouse antibody immobilized as a line in the control region
2. Directional Insert
3. (Optional) Single Specimen Collection Kit or equivalent) – or –

4. (Optional) Split Specimen Collection Kit or equivalent)
   Note: In addition to the materials supplied, a clock or other suitable timer is required.

WARNINGS & PRECAUTIONS

1. FOR *IN VITRO* DIAGNOSTIC USE ONLY.
2. For professional use only.
3. Urine samples have the potential to be infectious. Follow Universal Precautions for proper handling and disposal methods.
4. Do not use this kit beyond its expiration date.
5. This method was established using urine only. No other fluid has been evaluated.
6. Do not reuse the Test Device.

STORAGE & HANDLING REQUIREMENTS

Store at room temperature (15 – 28 °C); do not freeze. See expiration date for stability.

SAMPLE COLLECTION & PREPARATION

A fresh urine sample should be collected in one of the above-mentioned specimen collection kit or equivalent. Alternately, a clean, dry plastic or glass container, unused and without preservatives, may be used for specimen collection. Testing requires only a small volume (1 – 2 mL) of urine in the sample container. If required by your procedure, aliquot a portion of urine into the split sample container for later confirmation of results. If not required, dispose of all but 1 – 2 mL of urine and save the remainder for the test. Samples may be tested immediately or stored for up to 48 hours at 2 – 8 °C. For longer storage, freeze samples at –20 °C or below.

ASSAY PROCEDURE

PREPARATION

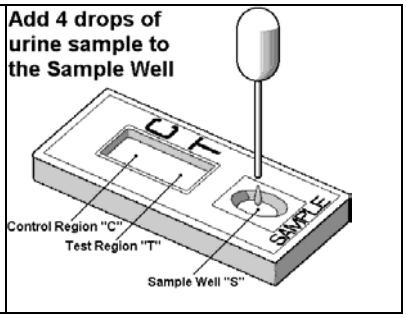
1. Confirm that all samples and test components are at room temperature (15 – 28 °C) before testing.
2. Do not break the seal on the foil pouch until you are ready to perform the test.

TESTING

1. Open the foil pouch at the notch and remove the test device and transfer pipette. Take care not to touch the exposed membrane. Place the device on a clean, level surface.

2. Hold the dropper vertically and dispense four (**4**) full drops of urine into the Sample Well. Wait five (**5**) seconds between adding each drop.

3. Read the result immediately at ten (**10**) minutes. Results read after more than 10 minutes have elapsed should be considered invalid.

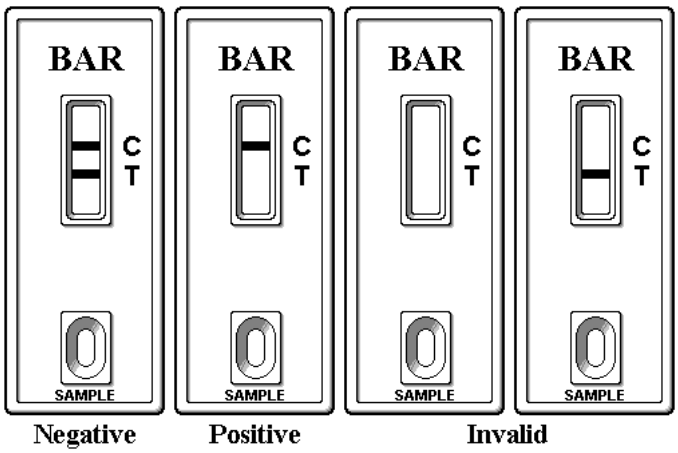


INTERPRETATION OF TEST RESULTS

Negative – A negative result is indicated when two (**2**) colored bands appear, one in the Control Region (**C**) and one in the Test Region (**T**). This result indicates a Barbiturate level that is below the detection sensitivity of 200 ng/mL.

Positive – A positive result is indicated when only one (**1**) colored band appears in the Control Region (**C**) and no band appears in the Test Region (**T**). This result indicates a Barbiturate level that is at or above the detection sensitivity of 200 ng/mL.

Invalid – A test must be considered invalid if no bands appear or if a band appears in the Test Region without a Control Band. The presence of a Control Band is necessary to confirm assay performance.



QUALITY CONTROL

An internal procedural control line has been incorporated into the test device to help ensure proper kit performance and reliability. However, the use of external controls is recommended. Positive and negative controls within 25% of the cutoff concentration should produce the expected results. For positive controls, only one (**1**) colored band will appear in the Control Region (**C**), and no band will appear in the Test Region (**T**). For negative controls, two (**2**) colored bands will appear, one in the Control Region (**C**) and one in the Test Region (**T**).

LIMITATIONS OF THE PROCEDURE

1. The possibility exists that substances and factors not described in this directional insert may interfere with the test, causing false results (*e.g.*, technical or procedural error).
2. This test has been developed for testing urine samples only. The performance of this test using other specimens has not been substantiated.
3. Adulterated urine samples may produce erroneous results. Strong oxidizing agents such as bleach (hypochlorite) can oxidize drug analytes. If a sample is suspected of being adulterated, obtain a new sample.
4. All positive samples must be confirmed by another method. Gas chromatography / mass spectrometry (GC/MS) is the method of choice to confirm the presence and concentration of a drug in urine.
5. This test is a qualitative, competitive screening assay. It is not designed to determine the quantitative concentration of drugs or the level of intoxication.
6. Because is a competitive assay, no prozone effect is present.
7. Occasionally, samples containing target drug concentrations below the cutoff sensitivity for the test may produce a positive result.

PERFORMANCE CHARACTERISTICS

SENSITIVITY (CUTOFF)

The Barbiturates Test detects barbiturates at a cutoff concentration equivalent to 200 ng/mL of Secobarbital. An evaluation of 80 clinical urine samples was performed to demonstrate the sensitivity.

Sample, *n*	Concentration Range (ng/mL of Secobarb)	Observed vs Expected Results	Sensitivity	Comments
20	0 to 100	20/20	>99%	
10	101 to 150	10/10	>99%	
10	151 to 200	9/10	90%	Sample 94% of cutoff at 188 ng/mL
10	201 to 250	9/10	90%	Sample 111% of cutoff at 222 ng/mL
20	251 to 350	20/20	>99%	
10	351 to 400	10/10	>99%	

KIT COMPARISON

In an evaluation of 204 clinical urine samples at 3 separate laboratory sites, including 2 independent clinical laboratories was compared to the Emit II Barbiturates test using a 200-ng/mL cutoff. Compared with the Emit II assay, an agreement of 97.5% (199 of 204) was observed.

Agreement		Emit II Barbiturates	
		Positive (121)	Negative (83)
DRG	(+)	119	3[1]
Barbiturates	(–)	2[2]	80

[1] Samples exhibited pronounced peaks in the ion window for barbiturates, however, compound(s) not identified.
[2] 2 samples were reported as "borderline positive" (±) by technician. Faint test bands seen, but GC/MS negative.

Discrepant Results: GC/MS vs Screening Methods [1]

Sample	DRG	EMIT II	GC/MS	Resolution
A100263	Positive	Negative	Negative [1]	Negative for tested barbiturates
A100276	Positive	Negative	Negative [1]	Negative for tested barbiturates
A100277	Positive	Negative	Negative [1]	Negative for tested barbiturates

PRECISION

Eight clinical urine pools ranging in concentration from 26 to 375 ng/mL of Secobarbital, as confirmed by GC/MS analysis, were assayed once a day for twenty days with the DRG Barbiturates Test. Two technicians interpreted the results individually.

Clinical Sample	Concentration (ng/mL of Secobarb)	Percent of Cutoff	Tech 1 Results	Tech 2 Results	Total Correct	Percent Correct
1	26	13	20/20 negative	20/20 negative	40/40	100
2	90	45	20/20 negative	20/20 negative	40/40	100
3	124	62	20/20 negative	20/20 negative	40/40	100
4	175	87.5	20/20 negative	20/20 negative	40/40	100
5	243	122	20/20 positive	20/20 positive	40/40	100
6	251	126	20/20 positive	20/20 positive	40/40	100
7	322	161	20/20 positive	20/20 positive	40/40	100
8	375	188	20/20 positive	20/20 positive	40/40	100
TOTALS			**160/160**	**160/160**	**320/320**	**100**

CROSS-REACTING SUBSTANCES

The following structurally related compounds were spiked into normal human urine and found to cross-react with the DRG Barbiturates Test. The results are expressed as that amount of compound capable of producing a result equivalent to 200 ng/mL of Secobarbital.

Compound	Concentration	Compound	Concentration	Compound	Concentration
Alphenal	400 ng/mL	Butabarbital	25 ng/mL	Pentobarbital	25 ng/mL
Amobarbital	150 ng/mL	Butalbital	300 ng/mL	Phenobarbital	200 ng/mL
Aprobarbital	50 ng/mL	Butethal	75 ng/mL	Secobarbital	200 ng/mL
Barbital	25 ng/mL	5,5-Diallylbarbituric Acid	100 ng/mL	(±)-Thiopental	9,500 ng/mL

INTERFERING SUBSTANCES

The following compounds were spiked into normal human urine and tested for interference with the DRG Barbiturates Test. Unless noted, these compounds were tested to 100 µg/mL with no interference observed.

Acetoacetic Acid • Acetone • *N*-Acetylprocainamide • Acetylsalicylic Acid (Aspirin) • Albumin • Alprazolam[A] • Amantadine • (+)-Amethopterin • Amikacin • *dl*-Aminoglutethimide • Aminopyrine • Amitriptyline • Amoxicillin • *d*, *dl* & *l*-Amphetamine • Ampicillin • Apomorphine • (−)-Arterenol • *l*-Ascorbic Acid (Vitamin C) • *d*, *dl* & *l*-Aspartic Acid • Atropine • Barbituric Acid • Benzoic Acid • Benzoylecgonine • Benzphetamine • Benztropine Methane Sulfonate • Bilirubin • Bromazepam • Bromocriptine Mesylate • (+)-Brompheniramine • Cannabidiol • Cannabinol • Carbamazepine • Cephalexin • Chloramphenicol • Chlordiazepoxide • Chloroquine • (+) & (±)-Chlorpheniramine • Chlorpromazine • Chlorpropamide • Chlorprothixene • Cimetidine • Clemastine • Clomipramine • Clonazepam • Clonidine • Cocaine • Codeine • (−)-Cotinine • Creatinine • Cyclizine • Cyclobenzaprine • Cyclosporin A • Cyproheptadine • Desipramine • Desmethyldiazepam • Dextromethorphan • Diazepam • Diflunisal • Digoxin • Diphenhydramine • 4-Dimeth-ylaminoantipyrine • Diphenoxylate • 5,5-Diphenylhydantoin • Disopyramide • Doxepin • Doxylamine • (+) & (−)-*ψ*-Ephedrine • (+), (±) & (−)-Ephedrine • (±) & (−)-Epinephrine • Erythromycin • Estriol • Estrone-3-Sulfate • Ethosuximide • Ethyl-*p*-Aminobenzoate • Ethylenediaminete-traacetic Acid • 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) • Ethylmorphine[B] • Fenfluramine • Fenoprofen • Fentanyl[B] • Flunitrazepam • Flurazepam • Furosemide • Gentamicin • Gentisic Acid • *dl*-Glutethimide • Griseofulvin • Guaiacol Glyceryl Ester • Hemoglobin • Heroin[B] • Hexobarbital • Hydrochlorothiazide • Hydrocodone • Hydromorphone • *dl*-*β*-Hydroxybutyric Acid • *o*-Hydroxyhippuric Acid • 5-Hydroxyindole-3-Acetic Acid • 5-Hydroxy-indole-2-Carboxylic Acid • 11-Hydroxy-Δ^9-THC[C] • 3-Hydroxytyramine • Hydroxyzine • Imipramine • Indole-3-Acetic Acid • Indole-3-Butyric Acid • Indomethacin • (+), (±)& (−)-Isoproterenol • Isoxsuprine • Kanamycin • Ketamine • Ketoprofen • Labetalol • Levorphanol • Lidocaine • Lithium Carbonate • (±)-Lorazepam • Lormetazepam • Lysergic Acid Diethylamide[D] • Medazepam • Melanin • Meperidine • Mephentermine • Meprobamate • Mescaline • *dl*-Metanephrine • (±)-Methadone • (+)-Methamphetamine • Methaqualone • (S)-6-Methoxy-*α*-Methyl-2-Naphthaleneacetic Acid • 2-Methyl-3-(3,4-Dihydroxyphenyl)-*dl* & *l*-Alanine • (±)-3,4-Methylenedioxyamphetamine • (±)-3,4-Methylenedioxymethamphetamine • Methylphenidate • Methyprylon • Metoclopramide • (±)-Metoprolol • Morphine • Morphine-3-*β*-D-Glucuronide • Nafcillin • Nalorphine • Naloxone • Naltrexone • Naphazoline • *α* & *β*-Naphthaleneacetic Acid • Netilmicin • Niacinamide • Nialamide • Nicotinic Acid • Nifedipine • Nitrazepam • Nomifensine • Norcodeine • Nordoxepin[B] • Norethindrone • Normorphine[B] • 11-Nor-Δ^8 & Δ^9-THC-Carboxylic Acid[C] • Nortriptyline • Noscapine • Nylidrin • Orphenadrine • Oxalic Acid • Oxazepam • Oxycodone • Oxymetazoline • Papaverine • Penicillin G • Pentazocine • Phencyclidine • Phenelzine • Pheniramine • Phenothiazine • Phentermine • Phenylacetone • *l*-Phenylalanine • Phenylbutazone • *trans*-2-Phenylcyclopropylamine • *l*-Phenylephrine • (R)-(+)-*α*, (±)-*α* & *β*-Phenylethylamine • (±)-Phenylpropanolamine • Piroxicam • Potassium Chloride • Prazepam • Prednisolone • Primidone • Procainamide • Procaine • Prochlorperazine • Promazine • Promethazine • (+)-Propoxyphene • 2-Propylpenta-noic Acid • Protriptyline • Pyrilamine • Quinidine • Quinine • Ranitidine • Riboflavin • Salicylic Acid • (−)-Scopolamine • Sulindac • Temazepam • Terbutaline • Tetracycline • Tetraethylthiuram Disulfide • Δ^8 & Δ^9-Tetrahydrocannabinol • Tetrahydrozoline • Thebaine • Theophylline • Thioridazine • *cis*-Thiothixene • Tobramycin • Triamterene • Triazolam[B] • Trifluoperazine • Triflupromazine • *dl*-Trihexyphenidyl • Trimethobenzamide • Trimethoprim • Trimipramine • Triprolidine • Tyramine • Urea • Uric Acid • Vancomycin • (±)-Verapamil • Zomepirac

[A] No interference was observed when the compound was tested to 25 µg/mL.
[B] No interference was observed when the compound was tested to 10 µg/mL.
[C] No interference was observed when the compound was tested to 5 µg/mL.
[D] No interference was observed when the compound was tested to 2.5 µg/mL.

PH

Clinical samples were tested for pH effect. Samples with a pH range of 4.5 to 8.5 were found to perform as expected in this assay.

SPECIFIC GRAVITY

Clinical samples were tested for specific gravity effect. Samples were tested with specific gravities ranging from 1.002 to 1.040. No variation was noted in expected versus obtained results.

BIBLIOGRAPHY & SUGGESTED REFERENCES

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