



BARBITURATE

Rapid Test (BAR) strip

Introduction

The BAR Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Barbiturate in human urine at a cut-off concentration of 300 ng/mL which is only utilized for in vitro diagnostics (IVD). It merely provides a preliminary result, therefore, an alternative method with more specificity is recommended to be used to confirm the initial results, in which case, the most preferred method is gas chromatography-mass spectrometry (GC-MS). For substance abuse cases, clinical considerations must be taken into account, especially when test results are positive.

Summary

Barbiturates are a group of drugs that affect the central nervous system and have a sedative and hypnotic effect. Barbiturates are used for many cases such as treating insomnia, treating anger and restlessness, anesthesia, etc. Barbiturates are addictive and their excessive consumption causes dependence. By binding to GABA nerve receptors and increasing their activity, barbiturates facilitate the entry of chlorine ions and have an inhibitory effect. The effects of barbiturates are, in many ways, similar to the temporary effects of alcohol. Small amounts produce calmness and relax muscles. Large doses can cause intoxication and drowsiness. Intoxicated by larger doses, the patient loses balance and dizziness and loss of speech will occur. In the case of overdose, it also causes unconsciousness, coma, and death. This drug is taken orally in the form of capsules or tablets. The short-term dosage of this medicine is 400 mg per day for 2 to 3 months. Only a very small amount of the drug (less than 5%) is excreted through urine. The detection period of barbiturates in urine is 4 to 7 days.

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Function Method Basis

Rapid barbiturate urine test employs an immunochromatographic method based on competitive binding. Substances that may be found in urine specimen compete with ones metabolized by the liver to bind to antibodies. During the test, the urine specimen flows upward via capillary action. If the amount of barbiturate in the urine is below 300 ng/mL, it does not bind to the binding sites of the antibodies coated with gold nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the barbiturate on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 300 ng/mL, no colored line will appear in the T area, since all the binding sites of the barbiturate antibodies are occupied. So, if the presence of barbiturate in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains barbiturate amounts lower than the desired level in the test, a colored line will appear in this area. In order to have a procedural control, a colored line always appears in the area of the control line (C), indicating the appropriate volume of the specimen added to the membrane.

Compounds

The test strip contains anti-barbiturate monoclonal antibody particles and a combination of conjugated protein-barbiturate. The secondary antibody prepared in goat species is employed for the control line.

+ **Caution:** for in vitro diagnostic use only.

Do not use after the expiration date. The test must remain in its package until use. All specimens must be considered infectious and all precautions and safety measures for handling and use must be taken.

Storage

The package should be stored at room temperature or in refrigerator (2-30 °C). The test is valid only until the expiration date printed on the package. Keep from freezing.

Specimen Collection and Preparation

+ **Urine collection:** The urine specimen must be collected in a clean and dry container. It could be collected at any time of the day.

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+ **Specimen storage:** The urine specimen can be stored at 2-8 °C for more than 48 hours, and frozen for long-term storage at -20 °C. Frozen specimens must be thawed and mixed well before testing.

Contents and Required Supplies

Items found in the kit: + Brochure + Test strip

Required items not included in the kit: + Specimen collection container + Timer

User Manual

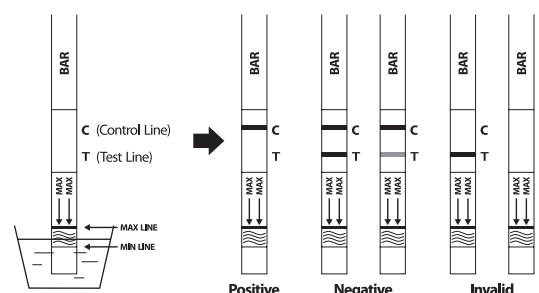
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

• Bring the pouch to room temperature, before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.

• Dip the test strip vertically into the urine specimen in the direction indicated by the arrows up to the MAX line and hold it for at least 10-15 seconds (See illustration).

• Place the test strip on a flat, non-absorbent surface, set the timer and wait for the colored line to appear. Interpret the results within 5 minutes.

+ Do not interpret the results after 10 minutes.



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Result Interpretation

Negative: Two lines appear, one line should be in the control area (C) and another colored line should appear in the test area (T). This negative result indicates that the barbiturate concentration is below the detectable level of 300 ng/mL.

+ **Attention:** The color shade in the testing area may vary, but it should be considered negative whenever there is even a faint colored line.

Positive: A colored line should appear in the control area (C) and no line should be seen in the test area (T). This positive result shows that the concentration of barbiturate is above the detectable level of 300 ng/mL.

Invalid: The control line (C) is not formed. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem is not solved, stop using the strip and contact the after-sales service immediately.

Quality Control

The quality control of the method is included in the test, and the colored line appearing in the control area (C) is considered as an inner controller which confirms the sufficient volume of the specimen and the correct execution of the test. The test strip does not come with control standards. It is recommended to do the test with positive and negative controls in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Barbiturate rapid test strip provides only preliminary analytical and qualitative results, and therefore, a secondary analytical method must be employed to confirm the results. For the confirmation of the results of this test, gas chromatography-mass spectrometry (GC-MS) is preferred.

2. There is a possibility of error in this technique. As some interfering substances in the urine specimen may lead to wrong results.

3. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, and should the substances remain, the test should be performed with another urine specimen.

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4. A positive result indicates the presence of substances or its metabolites. A negative result, on the other hand, may not necessarily indicate the absence of substances in the urine. The drug level may be lower than the level detectable by the test.

5. The test is not able to detect the abuse of certain therapeutic cases.

+ **Expected values:** if Barbiturate concentration is less than 300 ng/mL, the result will be negative and if the concentration is greater than 300 ng/mL, the result will be positive. The cut-off value of the test is 300 ng/mL.

Executive Feature

Accuracy: in order to compare the results of the Hana Gene's Rapid Barbiturate test kit adopting GC/MS method, 250 clinical specimens (102 positive and 148 negative) were collected, and Hana Gene's kit detected 98 specimens positive and 146 specimens negative. The results are presented in table below:

Method		GC/MS		Total Result
The BAR Rapid Test Dipstick	Results	Positive	Negative	
	Positive	98	2	100
	Negative	4	146	150
Total Result		102	148	250
Relative Sensitivity: 96.1%			Relative Specificity: 98.6%	
Total Agreement: 97.6%				

Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations from Secobarbital:

0 ng/mL, 150 ng/mL, 225 ng/mL, 300 ng/mL, 375 ng/mL, 450 ng/mL and 900 ng/mL. The results show 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below cut-off value. This information is summarized in the table below:

Secobarbital Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	27	3
300	Cut-off	30	15	15
375	+25%	30	3	27
450	+50%	30	0	30
900	3X	30	0	30

Analytical Features:

The following table shows the compounds positively identified by the barbiturate test strip in the urine specimen within 5 minutes.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Amobarbital	5,000	Cyclopentobarbital	30,000
5,5-Diphenylhydantoin	8,000	Pentobarbital	8,000
Allobarbitol	650	Alphenol	600
Barbital	8,000	Aprobarbital	500
Talbutal	200	Butabarbital	200
Butalbital	8,000	Butethal	500
Phenobarbital	300	Secobarbital	300

Precision

A study was carried out in three different centers by untrained people making use of three different lots of the product in order to evaluate the intra-rater, inter-rater, and inter-individual reliability.

A panel of coded specimens containing certain Secobarbital concentrations with GC/MS method was used in each center, at 0 ng/mL, 25% above and below the minimum detection limit, and 50% above and below the minimum detection limit of 300 ng/mL.

Secobarbital Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
150	10	10	0	10	0	10	0
225	10	9	1	8	2	9	1
375	10	2	8	1	9	2	8
450	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Secobarbital with concentrations of 150 ng/mL and 450 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain Secobarbital concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

Effect of Urinary pH

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given Secobarbital concentrations of 150 ng/mL and 450 ng/mL. The adjusted pH of the urine was done in a duplicate rapid barbiturate test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the interferences with positive and negative barbiturate test results, a study was carried out. The following compounds did not show any interference in the test results with 100 ng/mL barbiturate concentration:

Acetaminophenol	Diazepam	MDE	Phenylpropanolamine
Acetophenetidin	Diclofenac	Meperidine	Prednisolone
N-Acetylprocainamide	Diffunisal	Meprobamate	Prednisone
Acetylsalicylic acid	Digoxin	Methadone	Procaine
Aminopyrine	Diphenhydramine	L-Methamphetamine	Promazine
Amitypyline	Doxylamine	Methoxyphenamine	Promethazine
Amoxicillin	Ecgonine hydrochloride	(±) - 3,4-Methylenedioxy-amphetamine	D,L-Propranolol
Ampicillin	Ecgonine methylester	amphetamine	D-Propoxyphene
L-Ascorbic acid	(-) -ψ-Ephedrine	(±) - 3,4-Methylenedioxy	D-Pseudoephedrine
D,L-Amphetamine sulfate	[1R,2S] (-) Ephedrine	methamphetamine	Quinacrine
Apomorphine	L - Epinephrine	Morphine-3-β-D-glucuronide	Quinidine
Aspartame	Erythromycin	Morphine Sulfate	Quinine
Atropine	β-Estradiol	Nalidixic acid	Ranitidine
Benzilic acid	Estrone-3-sulfate	Naloxone	Salicylic acid

Benzoic acid	Ethyl-p-aminobenzoate	Naltrexone	Serotonin
Benzoyllecgonine	Fenoprofen	Naproxen	Sulfamethazine
Benzphetamine	Furosemide	Niacinamide	Sulindac
Bilirubin	Gentisic acid	Nifedipine	Temazepam
(±) - Brompheniramine	Hemoglobin	Norcodeine	Tetracycline
Caffeine	Hydralazine	Norethindrone	Tetrahydrocortisone
Cannabidiol	Hydrochlorothiazide	D-Norpropoxyphene	3-Acetate
Cannabinol	Hydrocodone	Noscapine	Tetrahydrocortisone
Chloralhydrate	Hydrocortisone	D,L-Octopamine	3-(β-D-glucuronide)
Chloramphenicol	O-Hydroxyhippuric acid	Oxalic acid	Tetrahydrozoline
Chlorothiazide	p-Hydroxyamphetamine	Oxazepam	Thiamine
(±) - Chlorpheniramine	p-Hydroxy-	Oxolinic acid	Thioridazine
Chlorpromazine	methamphetamine	Oxycodone	D,L-Tyrosine
Chlorquine	3-Hydroxytyramine	Oxymetazoline	Tolbutamide
Cholesterol	Ibuprofen	Papaverine	Triamterene
Clomipramine	Imipramine	Penicillin-G	Trifluoperazine
Clonidine	Iprenazid	Pentazocine hydrochloride	Trimethoprim
Cocacethylene	(±) - Isoproterenol	Perphenazine	Trimipramine
Cocaine hydrochloride	Isosuxiprine	Phencyclidine	Tryptamine
Codeine	Ketamine	Phenelzine	D,L-Tryptophan
Cortisone	Ketoprofen	Phentermine	Tyramine
(-) Cotinine	Labetalol	Trans-2-phenylcyclopropylamine hydrochloride	Uric acid
Creatinine	Levorphanol	propylamine hydrochloride	Verapamil
Deoxycorticosterone	Loperamide	L-Phenylephrine	Zomepirac
Dextromethorphan	Maprotiline	β-Phenylethylamine	

References

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