Domperidone
Doxylamine
Ecgonine
Ecgonine methylester
(+) Ephedrine
(±) Ephedrine
(–) Ephedrine
(–) Ψ Ephedrine
Epinephrine
Erythromycin
ß-Estradiol
Estriol
Estrone-3-sulfate
Ethyl-p-amino-benzoate
Fenoprofen
Furoxmide
Gentisic acid
Guaiacol glycerol ether
Glucose
Glucuronide
Glutethimide
Guaifenesin
Hemoglobin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketones
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Lysergic acid diethylaminde
Maprotiline
Melanin
Meperidine
Meprobamate
Methadone
D-Methamphetamine
p-Hydroxymetham-phetamine
Methaqualone
Methoxyphenamine
(±) 3,4-Methylene-dioxyamphet-amine
(±) 3,4-Methylene-dioxymeth-amphetamine
Methylphenidate
Methyprylon
Morphine
Morphine-3-ß-D-glucuronide
Nalidixic acid
Nalorphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
(–) Norpseudo-ephedrine
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
ß-Phenethylamine
Phenothiazine
Phentermine
Phenylbutazone
L-Phenylephrine
D-Phenylpropanol-amine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
D-Pseudoephedrine
L-Pseudoephedrine
Quinidine
Quinine
Rantidine
Salicylic acid
Serotonin
Sodium chloride
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Tetrahydrozoline
Δ^8-THC
Δ^9-THC
11-nor-Δ^9-THC-9-COOH
Thebaine
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L-Tryptophan
Tyramine
D,L-Tyrosine
Uric acid
Verapamil
Zomepirac

References

1. Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse.* Rockville, MD: National Institute on Drug Abuse (NIDA), Research Monograph 73;1986.
2. Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man.* 2nd Ed., Davis, CA: Biomedical Publ.;1982;p.488.

Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
PIP	Transfer Pipette
IFU	Instructions for Use
TEST DRUG	One-step immunochromatographic Assay for the Detection of Drugs of Abuse in Urine
TEST BAR	Barbiturates Test

EC REP
MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
Germany
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Manufactured by
PBM
Princeton BioMeditech Corporation
4242 US Hwy 1
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P-5845-F

AccuSign® BAR

One-Step Barbiturates Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Barbiturates and/or their Metabolites in Human Urine

PBM

Catalog No. DOA-206-35 35 Test Kit
DOA-206-10 10 Test Kit

Intended Use

The **AccuSign® BAR** test is a simple, one-step, immunochromatographic assay for the rapid, qualitative detection of barbiturates and/or their metabolites in human urine with a cutoff at 300 ng/mL for secobarbital.

The* AccuSign® BAR *test 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) is the preferred confirmatory method. Other chemical confirmatory methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.[1]

Summary and Explanation

Barbiturates are a group of chemicals derived from barbituric acid. Classified as hypnotics, they depress the central nervous system. Taken orally in pill or tablet form, they are prescribed for many medical conditions, usually for their sedative effect. Abuse of barbiturates can, however, lead not only to impaired motor coordination and mental disorder, but also to respiratory collapse, coma and death. The combination of barbiturates and alcohol is particularly dangerous.

Symptoms of barbiturate abuse include drowsiness, slurred speech and irritability. Acute conditions include respiratory collapse and loss of consciousness. Chronic conditions include addiction, abstinence, seizures, and death. The effects of short-acting barbiturates such as pentobarbital and secobarbital last 3 to 6 hours. The effects of long-acting barbiturates such as phenobarbital last 10 to 20 hours. Short-acting barbiturates normally remain detectable in urine for 4 to 6 days, while long-acting barbiturates can be detected for up to 30 days. Short-acting barbiturates are generally excreted as metabolites, while long-acting ones primarily appear unchanged.[1,2]

Principle

The **AccuSign® BAR** test uses solid-phase chromatographic membrane immunoassay technology for the qualitative detection of barbiturates. The test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition between the drug conjugates and the drugs which may be present in the urine sample, for binding to antibodies. In the test procedure, a sample of urine is placed in the Sample well of the device and is allowed to migrate upward. If the drug is present in the urine sample, it competes with the drug conjugate bound to the dye, for the limited antibodies immobilized on the membrane. If the level of drug or drug metabolite is above the cutoff level, the drug will saturate the antibodies, thus inhibiting the binding of the dye coated with drug conjugates to the antibodies on the membrane. This prevents the formation of a line on the membrane. Therefore, a drug-positive urine sample will not generate a line at the Test (T) position in the Result window, indicating a positive result from positive drug competition. A negative urine sample will generate a line at the Test position in the Result window, indicating a negative result from an absence of competition with free drugs.

In addition to the line that may appear at the Test position in the Result window, a Control line must appear at the Control (C) validation position in the Result window to confirm the viability of the test. This Control line should always be seen if the test is conducted properly. This works as a procedural control, confirming that proper sample volume was used and the reagent system worked. If insufficient sample volume is used, there may not be a Control line, indicating that the test is invalid.

Materials Provided

The **AccuSign® BAR** test kit contains all the reagents necessary to perform the assay.

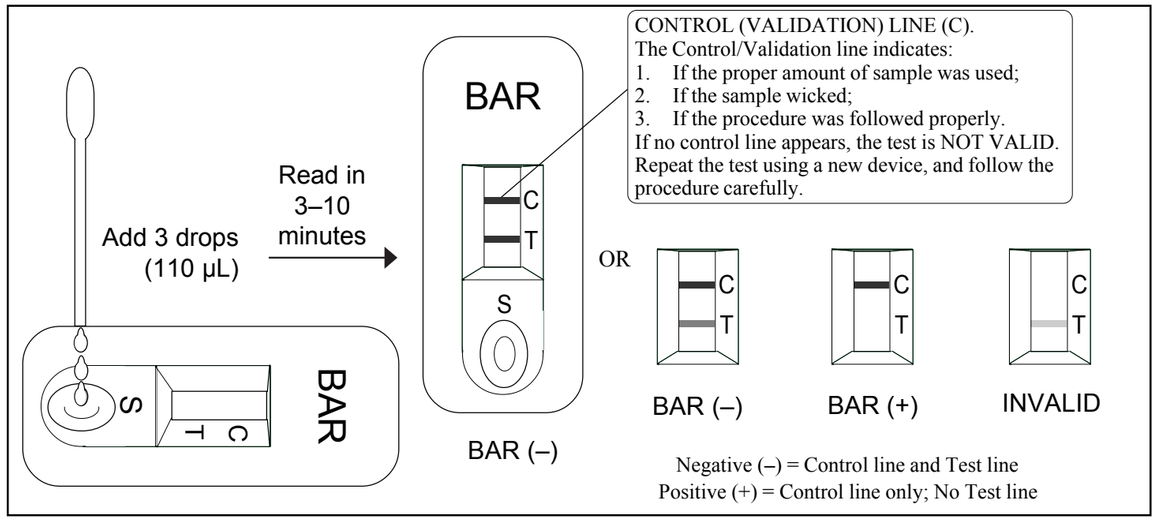
- **AccuSign® BAR** device. The test device contains a membrane coated with monoclonal anti-barbiturate antibody and a pad containing drug-dye conjugate in a protein matrix.
- Disposable sample dispenser.
- Instructions for use.

Precautions

- For *in vitro* diagnostic use only.
- Avoid cross contamination of urine samples by using a new urine specimen container and dropper for each urine sample.
- The test kit does not contain any HIV or hepatitis infective components.
- Urine specimens are potentially infectious. Proper handling and disposal methods should be established according to good laboratory practices.
- The **AccuSign® BAR** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.
- Do not use the test kit after the expiration date.

Storage and Stability

The **AccuSign® BAR** test kit should be stored at 2–30°C (35–86°F)



in the original sealed pouch. The expiration dating was established under these storage conditions.

Specimen Collection and Preparation

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed immediately, specimens should be refrigerated (2–8°C) or frozen. Specimens should be brought to room temperature before testing.

Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing to settle before testing.

Test Procedure

The test procedure consists of adding the urine sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. For each test, open one **AccuSign® BAR** pouch and label the **AccuSign® BAR** device with the patient ID.
2. Holding the dropper vertically, dispense 3 drops (110 µL) of the urine sample into the Sample well (**S**).
3. Read the result after 3 minutes, but within 10 minutes of sample addition.

Interpretation of Results

Negative: The appearance of a reddish-purple Control line (**C**) and a line next to T indicates a negative test result; i.e., no drug above the cutoff level has been detected. The color intensities of the Control line and the Test line may not be equal. *Any faint line next to T in the Result window, visible in 10 minutes, should be interpreted as negative. A negative test result does not indicate the absence of drug in the sample; it only indicates the sample does not contain drug above the cutoff level in qualitative terms.*

Positive: The appearance of only a reddish-purple Control line and no distinct line next to T indicates the test result is positive for BAR (i.e., the specimen contains BAR at a concentration above the cutoff level). *A positive test result does not provide any indication of the level of intoxication or urinary concentration of the drug in the sample; it only indicates the sample contains drug above the cutoff level in qualitative terms.*

Invalid: A distinct Control line (**C**) should always appear. The test is invalid if no Control line forms at the **C** position. Such tests should be repeated with a new **AccuSign® BAR** test device.

Limitations

- The test is designed for use with unadulterated urine only.
- There is a possibility that factors such as technical or procedural errors, as well as other substances in the urine sample which are not listed in Table 3 below, may interfere with the test and cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results. If adulteration is suspected, the test should be repeated with a new sample.
- The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.
- Certain medications containing barbiturates may produce a positive result.

User Quality Control

Internal Control: Each **AccuSign®** test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should always appear at the C position, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test has been performed correctly and the device is working properly, the background in the result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

The positive and negative procedural controls contained in each **AccuSign®** test device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear at the Control position, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing practice. For information on how to obtain controls, contact PBM's Technical Services.

Expected Values

AccuSign® BAR is a qualitative assay. The amount of barbiturates and/or their metabolites present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain barbiturates and/or their metabolites above the cutoff concentration.

Performance Characteristics

The **AccuSign® BAR** test has been shown to detect secobarbital at a cutoff of 300 ng/ml in urine. The test also detects other barbitals listed below at the minimum concentrations indicated (Table 2).

The accuracy of **AccuSign® BAR** was evaluated in comparison to a commercially available immunoassay (Syva® EMIT® II). A total of 248 samples was tested by both procedures. The overall accuracy of the test was 98.8%, as shown below (Table 1).

Table 1. Accuracy: Comparison of AccuSign® BAR with Syva® EMIT® II

		Syva® EMIT® II (BAR)		
		Positive	Negative	TOTAL
AccuSign® BAR	Positive	102	0	102
	Negative	3	143	146
TOTAL		105	143	248
		Relative Sensitivity	Relative Specificity	
AccuSign® BAR		97.1% (102/105)	> 99.9% (143/143)	

Discrepant samples for BAR were analyzed by GC/MS. The three false-negative samples contained the drug at 318, 304 and 306 ng/mL. In a separate study, **AccuSign® BAR** was evaluated with 23 specimens confirmed as positive by GC/MS. The range of drug values was 304 to 575 ng/mL. The results demonstrate the excellent correlation of **AccuSign® BAR** with GC/MS.

Precision and Accuracy

The precision of **AccuSign® BAR** was determined by carrying out the test with serially diluted standard drug solutions, using 3 lots of product on 3 different dates. About 98% of the samples containing secobarbital levels 25% over the cutoff level (i.e., 375 ng/mL) consistently showed positive results.

Distribution of Random Error:

Twenty (20) blind samples prepared by spiking various concentrations of secobarbital were separately tested by two operators. The test results from the two operators showed complete agreement.

Reproducibility

The reproducibility of the test results of **AccuSign® BAR** was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (600 ng/mL secobarbital), and 5 strongly positive samples (1,200 ng/mL secobarbital). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

Compounds that are detected by the **AccuSign® BAR** test are listed below (Table 2). The specificity of **AccuSign® BAR** was determined by adding various drugs and drug metabolites to drug-negative urine specimens and testing with the **AccuSign® BAR** test kit. The results are expressed in terms of the concentration required to produce a positive result.

Table 2. Specificity

Compound	Concentration (ng/mL)
Allobarbital	400
Alphenal	250
Amobarbital	5,000
Aprobarbital	400
Barbital	1,500
Butalbital	800
Cyclopentobarbital	400
Pentobarbital	2,000
Phenobarbital	5,000
Penytoin	4,000
Secobarbital	300
Thiopental	>100,000

The following compounds show no cross-reactivity when tested with **AccuSign® BAR** at a concentration of 100 µg/mL (Table 3).

Table 3. Non Cross-Reacting Compounds

Acetaldehyde	Aspartame	Chlorquine
4-Acetamidophenol	Atropine	Cholesterol
Acetone	Benzilic acid	Clomipramine
Acetophenetidin (Phenacetin)	Benzocaine	Clonidine
	Benzoic acid	Cocaine
N-Acetylprocainamide	Benzoylecgonine	Codeine
	Benzphetamine	Cortisone
Acetylsalicylic acid	Bilirubin	(–) Cotinine
Albumin	Caffeine	Creatinine
Aminopyrine	Calcium hypochloride	Deoxycorticosterone
Amitryptyline	Cannabidiol	Dextromethorphan
Amoxapine	Cannabinol	Dextropropoxyphene
Amoxicillin	Chloralhydrate	Diazepam
D,L-Amphetamine	Chloramphenicol	Diclofenac
L-Amphetamine	Chlordiazepoxide	Diethylpropion
Ampicillin	Chlorothiazide	Diflunisal
Apomorphine	Chlorpheniramine	Digoxin
Ascorbic acid	Chlorpromazine	Diphenhydramine