

SERATEC® Drug Screen BAR
REF DSH65

A visual one-step immunoassay for the qualitative detection of barbiturates in human urine. For professional *In Vitro* diagnostic use only.

INTENDED USE

The SERATEC Drug Screen BAR is a lateral flow, one-step immunoassay for the qualitative detection of barbiturates in human urine at a cut-off of 300 ng/ml (Secobarbital). This product is used to obtain a visual, qualitative result and is intended for professional use. The assay should not be used without proper supervision and is not intended for over the counter sale to lay persons.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/ MS) has been established as the preferred confirmatory method by the National Institute of Drug Abuse (NIDA). Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

BACKGROUND

Barbiturates are a class of central nervous system depressants that are chemically derived from barbituric acid. Clinically they are used as hypnotics, sedatives, narcotics and anti-convulsants.

Barbiturates are classified as short-acting and long-acting compounds. Their biological half-lives are highly variable ranging between 2 and 40 hours. Phenobarbital is an example of a long-acting barbiturate that has been used as daytime sedative and very extensively as an anti-convulsant. Pentobarbital and Secobarbital are two examples of short acting barbiturate hypnotics. Short acting barbiturates will generally be excreted in urine as metabolites, while the long-acting barbiturates will primarily appear unchanged.

A prolonged use of barbiturates may result in addiction. Withdrawal symptoms that occur after discontinued use may include insomnia, anxiety, convulsions and delirium. Frequently opiate or alcohol addicts additionally take barbiturates. The abuse of barbiturates can lead to an impairment of motor coordination and mental disorder. With time a tolerance to the sedative effects of the drug develops that results in an increase of drug intake. As the tolerance to the toxic effects of the drug does not develop in the same way an accidental (or willful) overdose of barbiturates may lead to respiratory collaps, coma or even death.

Urine based screening tests for drugs of abuse range from simple immunoassay tests to complex analytical procedures. The speed

and sensitivity of immunoassays have made them the most widely accepted method for screening urine for drugs of abuse. The SERATEC Drug Screen BAR is based on the principle of the highly specific immunochemical reactions of antigens and antibodies which are used for the analysis of specific compounds in biological fluids. This test is a rapid, visual, competitive immunoassay that can be used for the qualitative detection of barbiturates in human urine at a cut-off concentration of 300 ng/ml secobarbital. To review the amounts of the other structurally related compounds that are detected by the test, please see SPECIFICITY (back page).

PRINCIPLE

The SERATEC Drug Screen BAR is a one-step immunoassay in which a chemically labeled drug (drug conjugate) competes with the drug that may be present in urine for limited antibody binding sites. The test device contains a membrane strip, which was pre-coated with drug conjugate on the test band. A colored anti-barbiturate monoclonal antibody-colloidal gold conjugate pad is placed at the right end of the membrane. In the absence of drug in the urine, the solution of the colored antibody-colloidal gold conjugate and urine moves upward, chromatographically by capillary action, across the membrane. This solution migrates to the immobilized drug conjugate zone on the test band region. The colored antibody-colloidal gold conjugate attaches to the drug conjugate to form a visible line as the antibody complexes with the drug conjugate. Therefore, the formation of a visible precipitant in the test zone occurs, when the test urine is **negative** for the drug. When the drug is present in the urine, the drug/metabolite antigen competes with the drug conjugate on the test band region for limited antibody sites on the anti-barbiturate monoclonal antibody-colloidal gold conjugate. When a sufficient concentration of drug is present, it will fill the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate to the drug conjugate zone on the test band region. Therefore, absence of the color band on the test region indicates a **positive** result.

A control band with a different antigen/antibody reaction is also added to the immunochromatographic membrane strip at the control region (C) to indicate that the test has performed properly. This control line should always appear, regardless of the presence of drug and metabolite. This means that **negative** urine will produce **two** colored

bands, and **positive** urine will produce only **one** band. The presence of this colored band in the control region also serves as 1) verification that sufficient volume has been added, and 2) that proper flow was obtained.

STORAGE AND STABILITY

The test kit is to be stored refrigerated or at room temperature +4-+30 °C (38-86 °F) in the sealed pouch for the duration of the shelf life.

PRECAUTIONS

- For single *in-vitro* diagnostic use.
- For professional use only
- Urine specimens may be potentially infectious. Proper handling and disposal methods should be established.
- Avoid cross-contamination of urine samples by using a new specimen collection container and specimen pipette for each urine sample.
- Do not use test device if the pouch is damaged
- The components of the test of animal origin (e.g. antibodies) do not cause any danger if the test is used according to the instructions.

MATERIALS SUPPLIED IN THE KIT

- Test devices with disposable pipettes
- One instruction sheet

MATERIALS REQUIRED

- Specimen collection container
- Timer

SPECIMEN COLLECTION AND HANDLING

The SERATEC Drug Screen BAR is formulated for use with urine specimens. Fresh urine does not require any special handling or pre-treatment. Urine samples should be collected such that testing can be performed as soon as possible after the specimen collection, preferably during the same day. The specimen may be refrigerated at 2-8°C for 2 days, or frozen at -20°C for a longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Specimens previously frozen must be thawed, equilibrated to room temperature, and mixed thoroughly prior to testing.

Note: Urine specimens and all materials coming in contact with them should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

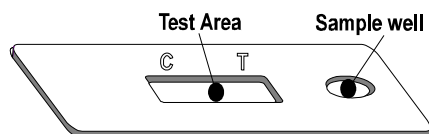
TEST PROCEDURE

Review "Specimen Collection" instructions. Test device, patient's samples, and controls should be brought to room temperature (20-30°C) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the test device from its protective pouch (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Label the device with patient or control identification.

2. Draw the urine sample to the line marked on the pipette (approximately 0.2 ml). Dispense the entire contents into the sample well. Use a separate pipette and device for each sample or control.

3. Read result between **3 to 8 minutes** after the addition of sample. Do not read result after 8 minutes.



INTERPRETATION OF RESULTS

Negative result:

Two colored lines appear in the viewing window. The line in the test region (T) is the drug probe line; the line in the control region (C) is the control line, which indicates proper performance of the device. The color intensity of the test line may be weaker or stronger than that of the control line.

Note: A weak test line indicates that the barbiturate concentration is close to the cut-off level. In this case the test should be repeated or the urine sample should be tested with a more specific method.

Positive result

Only **one** colored line appears in the control region (C). The **absence** of a test line indicates a positive result.

Invalid:

If no line appears in the control region the test is invalid and should be repeated



LIMITATIONS OF PROCEDURE

- The assay is designed for use with human urine only.
- A positive result with the test indicates the presence of a drug/metabolite only and does not indicate or measure intoxication.
- There is a possibility that technical or procedural errors as well as other substances and factors not listed (see SPECIFICITY) may interfere with the test and cause false results.
- If it is suspected that the samples have been mislabeled or tampered with, a new specimen should be collected.

QUALITY CONTROL

Good laboratory practice recommends the use of control materials to ensure proper kit

performance. Quality control specimens are available from commercial sources. When testing the positive and negative controls, use the same assay procedure as with a urine specimen.

PERFORMANCE CHARACTERISTICS*

*to adjust the concentration of barbiturate in the non-clinical samples the Sigma Drug Standard S4006 was diluted into drug-free human urine.

A. Accuracy

The accuracy of the SERATEC Drug Screen BAR was evaluated in comparison to a commercially available immunoassay. 121 urine samples, collected from presumed non-user volunteers, have been tested as negatives by both procedures with 100% agreement.

In a separate study, 73 urine samples were obtained from a clinical laboratory, where they had been screened and confirmed as positive for at least 1 of 5 different barbiturate derivatives (Amobarbital, Butalbital, Pentobarbital, Phenobarbital, Secobarbital) by GC/MS. They were tested with both immunoassays. With 42 samples, having barbiturate concentrations above the respective cut-offs, both tests showed positive results. 31 samples with Butalbital concentrations < 2000 ng/ml were determined negative with both tests. 2 samples were tested positive with both tests although their content of butalbital or pentobarbital, respectively, was lower than the detection limit. Here the presence of other barbiturate derivatives that had not been included in the GC/MS analysis could not be ruled out.

With the data obtained from the clinical specimens the performance characteristics of the test were calculated:

Diagnostic sensitivity:	100 %
Diagnostic specificity:	98.7 %
Positive predictive value:	94.9 %
Negative predictive value:	100 %
Reproducibility:	99.0 %

B. Reproducibility

The reproducibility of the SERATEC Drug Screen BAR test was evaluated at four different sites using blind controls. 60 of the samples containing 150 ng/ml secobarbital showed negative results including 8 (+/-) results showing a very faint test line. 60 samples with secobarbital concentrations of 600 ng/ml were determined as positive. Of the 60 samples containing secobarbital at the cut-off level of 300 ng/ml 5% tested positive and 95% were determined as (+/-), showing a very faint test line.

C. Precision

The precision of the test was determined with blind controls of the following secobarbital concentrations: 150; 225; 375; 450 ng/ml, respectively.

Conc. (ng/mL)	# samples	correct results	in %
150	50	50 (-)	100
225	50	50 (-) ¹	100
375	50	45 (+) ²	90
450	50	50 (+)	100

1: including 6 (+/-) results 2: the remaining 5 tests showed (+/-) results

D. Specificity

The specificity for the SERATEC Drug Screen BAR was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine.

The following structurally related compounds produced positive results when tested at levels equal to or greater than the concentrations listed below.

compound	concentration (ng/mL)
Allobarbital	1,000
Alphenal	300
Amobarbital	1,000
Aprobarbital	300
Barbital	300
Butalbital	300
Butethal	300
Butalbital	2,000
Pentobarbital	300
Phenobarbital	300
Secobarbital	300*

* „cut-off“

The following compounds were found not to cross-react when tested at concentrations up to 100 µg/ml.

Acetaminophen, Acetone, Albumin, Amitriptyline, D-Amphetamine, L-Amphetamine, Ampicillin, Aspartame, Aspirin, Atropine, Benzocaine, Benzoylcegonine, Bilirubin, (+)-Brompheniramine, Chloroquine, (+/-) Chlorpheniramine, Chlorpromazine, Cocaine, Codeine, Caffeine, Creatine, (-)-Deoxyephedrine, Dextromethorphan, Diazepam, 4-Dimethylaminoantipyrine, Dopamine, Doxylamine, Ecgonine, Ecgonin-methylester, (+/-)-Ephedrine, (+)-Epinéphrine, Erythronycin, Ethanol, Furosemide, Glucose, Guaiacol-Glycerol-Ether, Hemoglobin, Hydrocodone, Hydromorphone, Imipramine, (+/-) Isoproterenol, Lidocaine, Meperidine, Methadone, Methamphetamine, Metha-qualone, (1R,2S)-(-)-N-Methyl-Ephedrine, (+/-)-3,4-Methylenedioxyamphetamine, Methylphenidate, Morphine, Naloxone, Naltrexone, (+)-Naproxen, (+/-)-Norephedrine, Nortriptyline, Oxalic Acid, Oxazepam, Oxycodone, Penicillin-G, Pentemine, Phencyclidine, Pheniramine, Pheno-thiazine, L-Phenylephrine, Phenylethylamine, Procaine, Promethazine, D-Propoxyphene, Quinidine, Ranitidine, Sodium Chloride, Sulindac, 11-Nor- Δ^9 -THC-9-carboxylic acid, Thioridazine, Trifluoroperazine, Trimethobenzamide, Tyramine, Vitamin C

SUGGESTED READING

1. Baselt, R.C. Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, 1982
2. Urine Testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA), Research Monograph 73, 1986
3. Fed. Register, Department of Health and Human Services, Mandatory Guidelines for Federal Workplace Drug Testing Programs, 53, 69, 11970, 1988
4. McBay, A.J. Clin. Chem. 33, 33B-40B, 1987
5. Gilman, A.G., & Goodman, L.S. The Pharmacological Basis of Therapeutics, eds. MacMillan Publishing, New York, NY, 1980.

March 2008

