

## SERATEC® Drug Screen AMP REF DSA76

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

### INTENDED USE

The SERATEC Drug Screen AMP is a lateral flow, one-step immunoassay for the qualitative detection of amphetamine in human urine at a cut-off of 1000 ng/ml. 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

Amphetamine is a sympathomimetic phenethylamine derivative that prominently stimulates the central nervous system. The compound has been used in the treatment of obesity, narcolepsy and hypotension. D-amphetamine is 3-4 times more potent than the l-form.

Because of its stimulant effects amphetamine and structurally related compounds like metamphetamines are frequently abused. The drug may be self-administered either orally, by intravenous injection or by smoking. Moderate doses of amphetamine may result in euphoria, a feeling of increased energy and alertness, and insomnia. This is generally accompanied by a suppression of the appetite and an increase of the heart rate and the blood pressure. Some individuals become anxious, irritable and aggressive. Few may experience drowsiness. Higher doses may cause visual, auditory and tactile hallucinations that are sometimes accompanied by a paranoid psychosis that resembles a schizophrenic reaction. Cardiac dysrhythmias, hypertension, hyperpyrexia, convulsions and shock symptoms that might be followed by death due to respiratory and cardiac failure has been observed. Some studies indicate that heavy abuse may result in permanent damage to certain essential nerve structures in the brain.

Amphetamine is excreted with the urine either unchanged or after deactivation in the liver. The rate of excretion and the fraction of unchanged drug are dependent on the pH of the urine, increasing if the urine is acidic. The half-life is around 12 hours. As metamphetamine is metabolized partly to

amphetamine the detection of amphetamine in the urine indicates the consumption of amphetamine/metamphetamine within the previous 1-2 days.

Continuous abuse of amphetamine results in a tolerance to the euphorogenic effects of the drug that leads to higher and more frequent doses. Abrupt discontinuation of the use generally causes a mild form of withdrawal symptoms including fatigue, hyperphagia, craving for the drug and depression.

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 AMP 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 amphetamine in human urine at 1000 ng/ml cut-off concentration.

### PRINCIPLE

The SERATEC Drug Screen AMP is a one-step immunoassay in which a chemically labeled drug (drug conjugate) competes with the drug which 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-amphetamine 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-amphetamine 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 AMP 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.

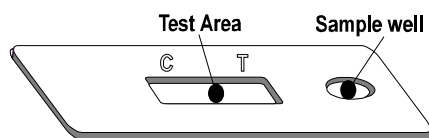
### TEST PROCEDURE

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 amphetamine 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 amphetamine in the non-clinical samples the Sigma Drug Standard A 3278 was diluted into drug-free human urine.

#### A. Accuracy

The accuracy of the SERATEC Drug Screen AMP was evaluated in comparison to a commercially available immunoassay at a cut-off of 1000 ng/ml. 120 urine samples, collected from presumed non-user volunteers, have been tested as negative by both procedures with 100% agreement.

In a separate study, 70 urine samples, obtained from a clinical laboratory where they were screened and confirmed as positive by the commercially available immunoassay and by GC/MS, were tested with the SERATEC Drug Screen AMP. The concentration of amphetamine in the urine samples ranged from 641 ng/ml to 4678 ng/ml. 48 samples with amphetamine concentrations  $\geq 1,500$  ng/ml, were found to be positive with the SERATEC test (100% agreement). Of 9 samples with amphetamine concentrations from 1,195 to 1,452 ng/ml, 7 were determined as positive and 2 as negative (+/-). 13 samples with amphetamine concentrations from 641 to 828 ng/ml were identified as negative by the SERATEC Drug Screen AMP.

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

Diagnostic sensitivity:	96.5 %
Diagnostic specificity:	100 %
Positive predictive value:	100 %
Negative predictive value:	98.5 %
Reproducibility:	98.9 %

#### B. Reproducibility

The reproducibility of the SERATEC Drug Screen AMP test was evaluated at four different sites using blind controls. 60 of the samples containing 500 ng/ml amphetamine showed negative results. 60 samples with amphetamine concentrations of 2000 ng/ml were determined as positive. Of the 60 samples containing amphetamine at the cut-off level of 1000 ng/ml 5% tested positive, 5% tested negative and 90% were determined as (+/-), showing a very faint test line.

### C. Precision

The precision of the test was determined with blind controls of the following amphetamine concentrations: 500; 750; 1250; 1500 ng/ml, respectively.

Conc. (ng/mL)	# samples	correct results	in %
500	50	50 (-)	100
750	50	50 (-) <sup>1</sup>	100
1250	50	32 (+) <sup>2</sup>	64
1500	50	50 (+)	100

1: including 19 (+/-) results 2: the remaining 18 tests showed (+/-) results

### D. Specificity

The specificity for the SERATEC Drug Screen AMP 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)
D-Amphetamine	1,000
L-Amphetamine	10,000
(+/-)3,4-MetylenDioxy-amphetamine (MDA)	5,000

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

Acetaminophen, Acetone, Albumin, Amitriptyline, Ampicillin, Aspartame, Aspirin, Atropine, Benzocaine, Bilirubin, Caffeine, Chloroquine, (+)-Chlorpheniramine, (+/-)-Chlorpheniramine, Creatine, Dexbrompheniramine, Dextromethorphan, 4-Dimethylaminoantipyrine, Dopamine, (+/-)-Ephedrine, (-)-Ephedrine, (+)-Epinephrine, Erythromycin, Ethanol, Furosemide, Glucose, Guaiacol-Glyceryl-Ether, Hemoglobin, Imipramine, (+/-)-Isoproterenol, Lidocaine, D-Methamphetamine, L-Methamphetamine, (1R,2S)-(-)-N-Methyl-Ephedrine, (+/-) 3,4-Methylenedioxy-methamphetamine, (+)-Naproxen, (+/-)-Norephedrine, Oxalic Acid, Penicillin G, Pheniramine, Phenothiazine, L-Phenylephrine,  $\beta$ -Phenylethylamine, Procaine, Quinidine, Ranitidine, Riboflavin, Sodium Chloride, Sulindac, Thioridazine, Trifluoroperazine, Trimethobenzamide, Tyramine, Vitamin C

### SUGGESTED READING

- Baselt, R.C. Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, 1982
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- McBay, A.J. Clin. Chem. 33, 33B-40B, 1987
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