DRG Amphetamine Test (RAP-2835)

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INTENDED USE

The DRG One Step Amphetamine Test is a rapid, qualitative immunoassay for the detection of Amphetamine in urine. The cutoff concentration for this test is 1,000 ng/mL, as recommended by the Substance Abuse and Mental Health Services Administration (SAMHSA), formerly the U.S. National Institute of Drug Abuse (NIDA).

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 chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are observed.

SUMMARY & EXPLANATION OF THE TEST

Amphetamine and its metabolites are central nervous system stimulants whose pharmacological properties include alertness, wakefulness, increased energy, reduced hunger and an overall feeling of well being. Large doses and extended usage can result in higher tolerance levels and physiological dependency. Both *d*- and *l*- forms of Amphetamine are controlled substances.

Urine based screening tests for drugs of abuse range from simple immunoassay tests to complex analytical procedures. The sensitivity and rapidity of 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 specimens.

PRINCIPLES OF THE PROCEDURE

The DRG One Step Amphetamine Test is a competitive immunoassay that is used to screen for the presence of Amphetamine 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 Amphetamine in urine with a high degree of confidence.

In the procedure, urine is added to the test device in the 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 Amphetamine levels are below 1,000 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 Amphetamine levels are at or above 1,000 ng/mL, 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 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 © by a non-specific antibody-dye / conjugate reaction. This band serves as a built-in quality control device that demonstrates antibody recognition and reactivity and confirms that the test is complete.

REAGENTS & MATERIALS SUPPLIED

1. 25 test devices containing:
   a. Monoclonal anti-Amphetamine antibody / colloidal gold conjugate in a protein matrix containing 0.1% sodium azide coated in the sample path
   b. Amphetamine 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 is established using urine. 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. Refer to 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 at least ½-inch (50 – 60 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 ½-inch of urine and save the remainder for the DRG 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.

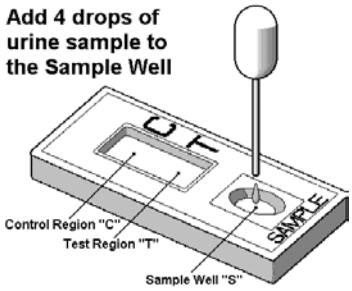
ASSAY PROCEDURE

Preparation

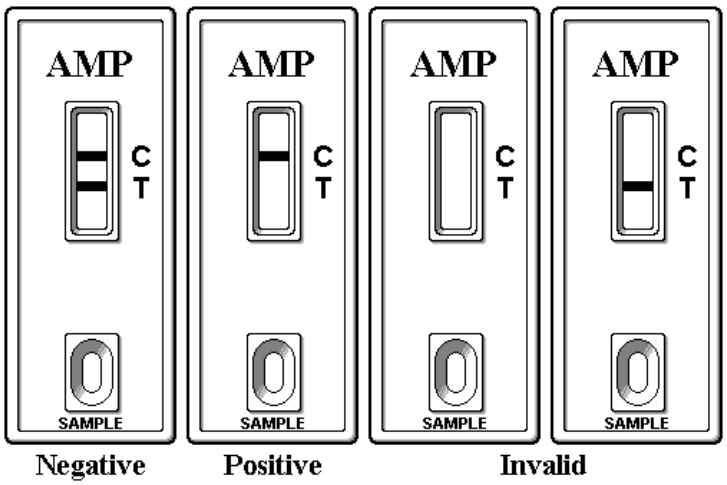
1. Confirm that all samples and test components are at room temperature (15 – 28 °C) before testing.
2. Do not break seal on the foil pouch until you are ready to perform the test.

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 © and one in the Test Region (**T**). This result indicates an Amphetamine level that is below the detection sensitivity of 1,000 ng/mL.
Positive – A positive result is indicated when only one (**1**) colored band appears in the Control Region © and no band appears in the Test Region (**T**). This indicates an Amphetamine level that is at or above the detection sensitivity of 1,000 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 ©, and no band will appear in the Test Region (**T**). For negative controls, two (**2**) colored bands will appear, one in the Control Region © 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 Amphetamine or the level of intoxication.
6. Because the DRG Test is a competitive assay, no prozone effect is present.
7. Occasionally, samples containing Amphetamine levels below the cutoff sensitivity for the test may produce a positive result.

PERFORMANCE CHARACTERISTICS

Sensitivity – The DRG Amphetamine Test detects Amphetamine and its major metabolites at a cutoff concentration equal to or greater than 1,000 ng/mL. The sensitivity of the DRG Test was evaluated on 189 urine samples and compared with both commercially available immunoassays and GC/MS. At a cutoff of 1,000 ng/mL, a sensitivity of 98% was observed.
Specificity – The specificity of the DRG™ Amphetamine Screening Test was evaluated on 104 urine samples and compared with commercially available immunoassays using the 1,000 ng/mL cutoff. In three separate laboratory studies, including two clinical trials, a specificity of >99% (104/104) was observed.
Accuracy – The accuracy of the DRG Amphetamine Screening Test was evaluated on 189 urine samples and compared with two commercially available immunoassays using the 1,000 ng/mL cutoff. An agreement of greater than 97% was observed. In addition, studies at two separate, independent clinical laboratories produced an agreement of >99% accuracy (123/124) when compared to the Emit II assay.

Precision – Eight urine pools ranging from 0 to 1,930 ng/mL were assayed twice a day for twenty days with the DRG Amphetamine Test. The results were interpreted individually by two technicians. The inter- and intra-assay coefficients of variation were <1% for all samples.

Cross-Reactivity – The following structurally related compounds were spiked into normal human urine and found to cross-react in the DRG Amphetamine Test. The results, in ng/mL, are expressed as that amount of compound capable of giving a result equivalent to 1,000 ng/mL Amphetamine.

Compound	Conc.	Compound	Conc.
d-Amphetamine	1,000	(±)-3,4-Methylenedioxyamphetamine	4,500
dl-Amphetamine	10,000	(±)-α-Phenylethylamine	10,000
l-Amphetamine	100,000	®-(+)-α-Phenylethylamine	100,000
3-Hydroxytyramine	10,000	β-Phenylethylamine	10,000
Mephentermine	100,000	Tyramine	12,500

Interfering Substances – The following compounds were spiked into normal human urine and tested for interference with the DRG™ Amphetamine Test. These compounds were tested to 100 µg/mL, unless otherwise noted, with no interference observed.

Acetaminophen • Acetone • *N*-Acetylprocainamide • Acetylsalicylic Acid (Aspirin) • Albumin • Alphenal • Alprazolam[A] • Amantadine • (+)-Amethopterin • Amikacin • *dl*-Aminoglutethimide • Aminopyrine • Amitriptyline • Amobarbital • Amoxicillin • Ampicillin • Apomorphine • Aprobarbital • (–)-Arterenol • *l*-Ascorbic Acid (Vitamin C) • Aspartame • *d*, *dl* & *l*-Aspartic Acid • Atropine • Barbital • Barbituric Acid • Benzoic Acid • Benzoylecgonine • Benzphetamine • Benztropine Methane Sulfonate • Bilirubin • Bromazepam • Bromocriptine Mesylate • (+)-Brompheniramine • Butabarbital • Butalbital • Butethal • Caffeine • 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 • (–)-Deoxyephedrine • Desipramine • Desmethyldiazepam • Dextromethorphan • 5,5-Diallylbarbituric Acid • Diazepam • Diflunisal • Digoxin • 4-Dimethylaminoantipyrine • Diphenhydramine • Diphenoxylate • 5,5-Diphenylhydantoin • Disopyramide • Doxepin • Doxylamine • (+) & (–)-ψ-Ephedrine • (+), (±) & (–)-Ephedrine • (±) & (–)-Epinephrine • Erythromycin • Estriol • Estrone-3-Sulfate • Ethanol • Ethosuximide • Ethyl-*p*-Aminobenzoate • Ethylenediaminetetraacetic Acid • 2-Ethylidene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) • Ethylmorphine[B] • Fenfluramine • Fenoprofen • Fentanyl[B] • Flunitrazepam • Flurazepam • Furosemide • Gentamicin • Gentisic Acid • Glucose • *dl*-Glutethimide • Griseofulvin • Guaiacol Glyceryl Ester • Hemoglobin, Human • Heroin[B] • Hexobarbital • Hydrochlorothiazide • Hydrocodone • Hydromorphone • *o*-Hydroxyhippuric Acid • 5-Hydroxyindole-3-Acetic Acid • 5-Hydroxyindole-2-Carboxylic Acid • 11-Hydroxy-Δ^9-THC[C] • Hydroxyzine • Ibuprofen • Imipramine • Indole-3-Acetic & Butyric Acid • Indomethacin • (+), (±) & (–)-Isoproterenol • Isoxsuprine • Kanamycin • Ketamine • Ketoprofen • Labetalol • Levorphanol • Lidocaine • Lithium Carbonate • (±)-Lorazepam • Lormetazepam • Lysergic Acid Diethylamide (LSD)[D] • Medazepam • Melanin • Meperidine • 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-Methylenedioxymethamphetamine • Methylphenidate • Methyprylon • Metoclopramide • (±)-Metoprolol • Morphine • Morphine-3-β-D-Glucuronide • Nafcillin • Nalorphine • Naloxone • Naltrexone • Naphazoline • α & β-Naphthaleneacetic Acid • Naproxen • 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 • Orphena-

drine • Oxalic Acid • Oxazepam • Oxycodone • Oxymetazoline • Papaverine • Penicillin G • Pentazocine • Pentobarbital • Phencyclidine • Phenelzine • Pheniramine • Phenobarbital • Phenothiazine • Phentermine • Phenylacetone • *l*-Phenylalanine • Phenylbutazone • *trans*-2-Phenylcyclopropylamine • *l*-Phenylephrine • (±)-Phenylpropanolamine • Piroxicam • Potassium Chloride • Prazepam • Prednisolone • Primidone • Procainamide • Procaine • Prochlorperazine • Promazine • Promethazine • (+)-Propoxyphene • 2-Propylpentanoic Acid • Protriptyline • Pyrilamine • Quinidine • Quinine • Ranitidine • Riboflavin • Salicylic Acid • (–)-Scopolamine • Secobarbital • Sodium Chloride • Sulindac • Temazepam • Terbutaline • Tetracycline • Tetraethylthiuram Disulfide (Antabuse) • Δ^8 & Δ^9-Tetrahydrocannabinol • Tetrahydrozoline • Thebaine • Theophylline • Thioridazine • *cis*-Thiothixene • Tobramycin • Triamterene • Triazolam[B] • Trifluoperazine • Triflupromazine • *dl*-Trihexyphenidyl • Trimethobenzamide • Trimethoprim • Trimipramine • Triprolidine • 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.

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