



AMPHETAMINE

Rapid Test (AMP) strip

Introduction

The AMP Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Amphetamine in human urine at a cut-off concentration of 500 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 high pressure liquid chromatography (HPLC).

Summary

Amphetamine (contracted from alpha-methylphenethylamine) is a central nervous system (CNS) stimulant which by affecting the brain's neurotransmitters, accelerates the brain-body communication, and is used in the treatment of attention deficit hyperactivity disorder (ADHD), obesity, and drug abuse.

The effects of Amphetamine on the brain and body include the faster release of chemical intermediates such as dopamine and norepinephrine from nerve terminals, increased body temperature and breathing, increased desire to speak, euphoria, decreased fatigue, reduced desire for food and so on. Long-term use of this drug results in unpleasant conditions such as severe mental states, skin diseases, vitamin deficiency, wounds, the possibility of sinking into a coma, cardiac arrhythmia, unusual weakness and fatigue, changes in behavior and social interests, breathing difficulties, increased heart rate, loss of balance between behavior and psychological personality.

This substance could be taken orally and via injection. Obviously, the longer the use, the more the dependence on it, leading to an increase in the dosage in the long run.

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The onset of action of Amphetamine is usually 2 to 4 hours after taking and its half-life in the body is around 4 to 24 hours. About 30% of the substance remains unchanged and the rest is excreted from the body through urine in a hydroxylated form.

Amphetamine test is a rapid urine screening test which could be used without any equipment. In this test, monoclonal antibody is used for the selective detection of increased levels of the substance in urine. The concentrations of Amphetamine greater than 500 ng/mL indicate that the test result is positive.

Function Method Basis

The Amp Rapid Test is an Immunochromatography method based on the principle of competitive binding. During the test, the urine specimen flows on the nitrocellulose membrane via capillarity action. If the Amphetamine level in the urine specimen is equal to or greater than 500 ng/mL, then it competes with the antibody conjugated with gold particles to bind to the coated antigen in the T area. And because all the binding terminals for the anti-Amphetamine antibodies are saturated, a colored line will not be formed in the test line region (T). If the level of Amphetamine in the urine is below 500 ng/mL, it does not saturate the binding terminals for the coated antibody particles. These particles coated with immobilized anti-Amphetamine are trapped and show a visible colored line in test line area (T). 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 coated Amphetamine with anti-Amphetamine antibodies conjugated with gold nanoparticles. The secondary antibody prepared in goat species is also 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 until the expiration date printed on the package. Keep from freezing. Do not use after the expiration date.

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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.

+ **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 specimen 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

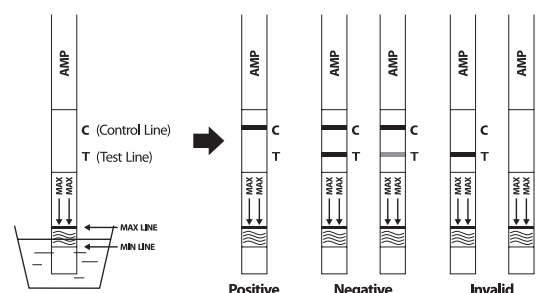
Before testing, allow the test strip and the urine specimen reach room temperature (15-30 °C).

• 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, turn on 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 the other colored line should appear in the test area (T). This negative result indicates that the Amphetamine concentration is below the detectable level of 500 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 Amphetamine is above the detectable level of 500 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: the colored line appearing in the control area (C) 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. Amphetamine 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, high pressure liquid chromatography (HPLC) 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. It is possible to add substances like bleaches or white alum in the urine specimen. Despite the analytical method used, it may cause a wrong result, 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 Amphetamine concentration is less than 500 ng/mL, the result will be negative and if the concentration is greater than 500 ng/mL, the result will be positive. The cut-off value of the test is 500 ng/mL.

Executive Feature

Accuracy: A comparative method was adopted using Hana Gene's Rapid Amphetamine test strip and an available commercial rapid Amphetamine test. The test was performed on 310 clinical specimens (132 positive specimens and 178 negative specimens), and Hana Gene's kit detected 132 specimens positive and 175 specimens negative. The results are presented in table below:

Method	other Rapid Test Result		Total Result
The AMP Rapid Test Dipstick	Results	Positive	Negative
	Positive	132	3
	Negative	0	175
Total Result		132	178

Sensitivity: > 99% (97.2 % ~ 100%) **Specificity:** 98.3% (95.1% ~ 99.6%)
Accuracy: 99.0% (97.2% ~ 99.8%) 95% Confidence Intervals

The comparison was made using the rapid Amphetamine test and TLC, The test was performed on 275 clinical specimens (120 positive specimens and 155 negative specimens) Hana Gene's kit detected 117 specimens positive and 150 specimens negative. results are shown in the table below.

Method	TLC		Total Result
The AMP Rapid Test Dipstick	Results	Positive	Negative
	Positive	117	5
	Negative	3	150
Total Result		120	155

Sensitivity: > 97.5% (92.8 % ~ 99.4%) **Specificity:** 96.7% (92.6% ~ 98.9%)
Accuracy: 97.0% (94.3% ~ 98.7%) 95% Confidence Intervals

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Analytical Sensitivity:

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

0 ng/mL, 250 ng/mL, 375 ng/mL, 500 ng/mL, 625 ng/mL, 750 ng/mL and 1500 ng/mL. The results show 99% accuracy above 50% and below 50% of cut-off value. This information is summarized in the table below:

Amphetamine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Positive	Negative
0	0	30	0	30
250	-50%	30	0	30
375	-25%	30	5	25
500	Cut-off	30	23	7
625	+25%	30	28	2
750	+50%	30	30	0
1500	3X	30	30	0

Analytical Features:

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

Compound
D,L-Amphetamine sulfate
L-Amphetamine
(±) 3,4-Methylenedioxymphetamine
Phentermine
Maprotiline
Methoxyphenamine
D-Amphetamine

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Precision

A study was carried out in three different sites (clinics) 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 Amphetamine concentrations at 0 ng/mL, 25% above and below the minimum detection limit, and 50% above and below the minimum detection limit of 500 ng/mL was used in each center.

Amphetamine Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
250	10	10	0	10	0	10	0
375	10	10	0	10	0	9	1
625	10	1	9	0	10	0	10
750	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Amphetamine with concentration of 250 ng/mL and 750 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain Amphetamine concentrations were checked by the test strip. The results show that the specific weight and gravity does 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 Amphetamine concentrations of 250 ng/mL and 750 ng/mL. The adjusted pH of the urine was done a duplicate rapid Amphetamine test whose results show that the pH range does not interfere with the test.

Cross Reactivity

The antibody used in this test has a high specificity, and according to the claim of the manufacturing company producing the antigens and antibodies, the following compounds did not show any interference in the test results:

4-Acetamidophenol Creatinine Loperamide Prednisone Acetophenetidin Deoxycorticosterone Maprotiline Procaine N-Acetylprocainamide Dextromethorphan Meperidine Promazine Acetylsalicylic acid Diazepam Meprobamate Promethazine Aminopyrine Diclofenac Methadone D,L-Propanolol Amitriptyline Diflunisal Methoxyphenamine D-Propoxyphene Amobarbital Digoxin (+) 3,4-Methylenedioxy- D-Pseudoephedrine Amoxicillin Diphenhydramine Amphetamine Quinacrine Ampicillin Doxylamine 3,4-Methylenedioxyethyl- Quinidine L-Ascorbic acid Ecgonine hydrochloride Amphetamine Quinine D-Amphetamine Ecgonine methyl ester Methylphenidate Ranitidine D,L-Amphetamine (1R,2S)-(-)-Ephedrine Morphine 3-D- Salicylic acid L-Amphetamine L-Epinephrine glucuronide Secobarbital Apomorphine (-)-ψ-Ephedrine Nalidixic acid Serotonin Aspartame Erythromycin Naloxone (5-Hydroxytyramine) Atropine β-Estradiol Naltrexone Sulfamethazine Benzilic acid Estrone-3-sulfate Naproxen Sulindac Benzoic acid Ethyl-p-aminobenzoate Niacinamide Temazepam Benzoyllecgonine Fenfluramine Nifedipine Tetracycline Benzphetamine Fenopropfen Norethindrone Tetrahydrocortisone, Bilirubin Furosemide D-Norpropoxyphene 3-Acetate-Brompheniramine Gentisic acid Noscapine Tetrahydrocortisone Caffeine Hemoglobin D,L-Octopamine 3-D glucuronide) Cannabidiol Hydralazine Oxalic acid Tetrahydrozoline Chloralhydrate Hydrochlorothiazide Oxazepam Thiamine Chloramphenicol Hydrocodone Oxolinic acid Thioridazine Chlordiazepoxide Hydrocortisone Oxycodone D, L-Tyrosine Chlorothiazide p-Hydroxyamphetamine Oxymetazoline Tolbutamine Chlorpheniramine O-Hydroxyhippuric acid Papaverine Trans-2-phenyl Chlorpromazine 3-Hydroxytyramine Penicillin-G cyclopropylamine Chlorquine Ibuprofen Pentobarbital Triamterene Cholesterol Imipramine Perphenazine Trifluoperazine Clomipramine Iproniazid Phencyclidine Trimethoprim Clonidine-Isoproterenol Phenelzine Trimipramine Cocaethylene Isoxsuprine Phenobarbital Tryptamine Cocaine hydrochloride Ketamine Phentermine D, L-Tryptophan Codeine Ketoprofen L-Phenylephrine Tyramine Cortisone Labetalol β-Phenylethylamine Uric acid (-) Cotinine Levorphanol Phenylpropanolamine Verapamil Prednisolone Zomepirac

References

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



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