



METHAMPHETAMINE

Rapid Test (MET) strip

Introduction

The MET Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Methamphetamine 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

Methamphetamine is an addictive drug, chemically similar to amphetamine, both of which causing the release of monoamine neurotransmitters (dopamine, serotonin, norepinephrine), however, Methamphetamine's effects on the central nervous system are greater. Such substances are considered important more because of the complications caused by their misuse. This drug could be taken orally or by injection. Obviously, frequent, prolonged use of it leads to dependence and eventually, higher doses. Ice/glass (crystal meth) is the strongest type of Methamphetamine which is produced illegally. Higher doses of this drug the central nervous system and induce a state of euphoria, satisfaction, alertness, decreased appetite, and a sense of increased energy and strength. Cardiovascular responses to Methamphetamine include increased blood pressure, and irregular heartbeat. More acute responses include anxiety, paranoia, hallucinations, psychotic behaviors, depressive moods, and fatigue. Usually, the effects of Methamphetamine are around 2 to 4 hours with a half-life of 9 to 24 hours in the body. It is excreted through urine in the form of amphetamine, oxidized compounds

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and amine-free derivatives, although 10-20% of Methamphetamine is excreted unchanged through urine. Therefore, the main compound in the urine indicates that Methamphetamine has been used. It is usually detectable in urine after 3 to 5 days depending on the level of urine pH. Methamphetamine test strip is a rapid urine screening method, in which monoclonal antibodies are employed for the selective detection of high levels of Methamphetamine in the urine. Should the level be greater than 500 ng/mL, the test result will be positive.

Function Method Basis

Rapid Methamphetamine urine test employs an immunoassay method based on competitive binding. If the analyte being tested is present in the urine specimen, it competes with the antibodies conjugated with gold nanoparticles to bind to the antigen binding site. During the test, the urine specimen flows upward via capillary action. If the amount of Methamphetamine in the urine is below 500 ng/mL, it does not bind to the binding sites of the antibodies. Consequently, the antibody conjugated with nanoparticles will be trapped by the stabilized Methamphetamine in the test area (T) and will leave a colored line there. Should the amount be equal to or greater than 500 ng/mL, no colored line will appear in the T area, since all the binding sites of the Methamphetamine antibodies are occupied. 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 Methamphetamine with anti-Methamphetamines 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 only until the expiration date printed on the package. Keep from freezing.

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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 specimens 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

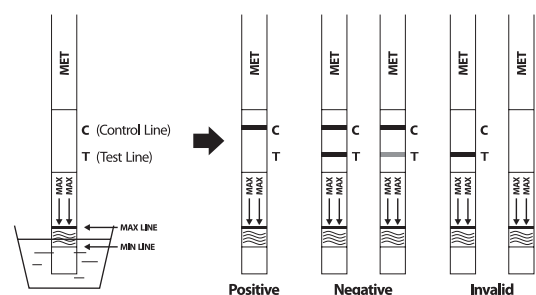
Before testing, allow the test strip and the urine specimen reach room temperature (18-30 °C).

• 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, set 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 another colored line should appear in the test area (T). This negative result indicates that the Methamphetamine 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 Methamphetamine 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, and the colored line appearing in the control area (C) is considered as an inner controller which 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. Methamphetamine 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. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, 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 their 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 Methamphetamine 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: The results obtained from Hana Gene's MET Rapid Test strip were compared with those of an available commercial kit. The test was performed on 280 clinical specimens, collected from individuals present for drug screening test. The results are presented in table below:

Method		Hana Gene Result		REF. Result
The MET Rapid Test Dipstick	Results	Positive	Negative	
	Positive	104	0	104
	Negative	1	175	176
Total Result		105	175	280

Sensitivity: 99.0% (94.8 % ~ 99.9%)

Specificity: > 99.0% (97.9% ~ 100.0%)

Accuracy: 96.6% (98.0% ~ 99.9%)

95% Confidence Intervals

In order to compare the results of Hana Gene's MET Rapid test kit, using TLC method, 235 clinical specimens (115 positive; 120 negative) were collected, and Hana Gene's kit detected 112 positive and 115 of the specimens negative. The results are shown in the table below:

Method		TLC		Total Result
The MET Rapid Test Dipstick	Results	Positive	Negative	
	Positive	112	5	117
	Negative	3	115	118
Total Result		115	120	235

Sensitivity: > 97.3% (92.5 % ~ 99.4%)

Specificity: 95.8% (90.5% ~ 98.6%)

Accuracy: 96.6% (93.4% ~ 98.5%)

95% Confidence Intervals

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Analytical Sensitivity:

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

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 more than 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below cut-off value. This information is summarized in the table below:

MetAmphetamine 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	7	23
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 Methamphetamine test strip in the urine specimen within 5 minutes.

Compound
±-Hydroxymethamphetamine
D-Methamphetamine
L-Methamphetamine
±-3,4-Methylenedioxymethamphetamine

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Precision

A study was carried out in three different centers 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 Methamphetamine concentrations was used in each center, 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.

MetAmphetamine Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
250	10	10	0	9	1	10	0
375	10	8	2	10	0	9	1
625	10	0	10	0	10	0	10
750	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Methamphetamine with concentrations 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 Methamphetamine concentrations were checked by the test strip. The results show that the specific weight and gravity do 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 Methamphetamine concentrations of 250 ng/mL and 750 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Methamphetamine test whose results show that the pH range does not interfere with the test.

Non Cross-Reacting Compounds

The antibody used in this test has a high specificity and according to the manufacturing company, the following compounds did not show any interference in the Methamphetamine 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 methylester 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. 488 ;1982
- Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 1986 ;73



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