



METHYLENEDIOXY-METHAMPHETAMINE Rapid Test (MDMA) strip

Introduction

The MDMA Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Ecstasy 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, qualitative and analytical 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 gas chromatography-mass spectrometry (GC-MS). In cases of drug abuse, especially when the test results are positive, clinical and expert observations are required.

Summary

Ecstasy or methylenedioxy-methamphetamine is a synthetic drug contracted from amphetamines and phenethylamines, which was first produced by a German pharmaceutical company in 1914 to treat obesity. MDMA causes the release of serotonin, dopamine, and noradrenaline in the brain thus being euphoric, considerably lowering the individual's anxiety level. MDMA abuse affect body's normal temperature and increases it, which causes dehydration due to excess perspiration. Of course, ecstasy makes the body secrete a hormone that stops urination, and so drinking a lot of water leads to salt imbalance in the body. These processes cause disorders and sometimes death. Ecstasy rapid test result becomes positive when the methylenedioxy-methamphetamine level in the urine exceeds 500 ng/mL.

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Function Method Basis

Rapid ecstasy urine test employs an immunochromatographic method based on competitive binding. Substances that may be found in urine specimen compete with ones metabolized by the liver to bind to antibodies. During the test, the urine specimen flows upward via capillary action. If the amount of ecstasy in the urine is below 500 ng/mL, it does not bind to the binding sites of the antibodies coated with gold nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the ecstasy on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 500 ng/mL, no colored line will appear in the T area, since all the binding sites of the ecstasy antibodies are occupied. So, if the presence of ecstasy in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains ecstasy amounts lower than the desired level in the test, a colored line will appear in this area. 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 particles bound to anti-ecstasy monoclonal antibody particles and a combination of conjugated protein-ecstasy. 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.

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.

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+ 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

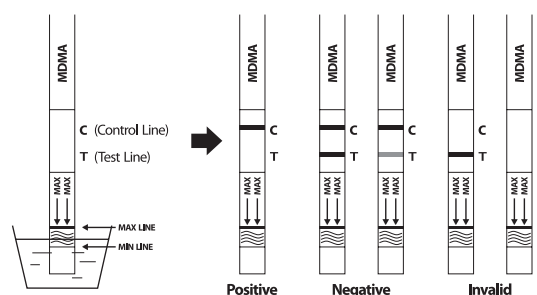
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

• 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 ecstasy 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 ecstasy 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. Ecstasy 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, gas chromatography-mass spectrometry (GC-MS) 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 the substance or its metabolites. A negative result, on the other hand, may not necessarily indicate the absence of the substance 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 ecstasy 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 MDMA Rapid Test strip were compared with those of an available commercial kit. In a study on 110 urine specimens (48 positive and 62 negative), Hana Gene's kit detected 48 of the specimens positive and 62 negative. The results are presented in table below:

Method		other MDMA Rapid Test		Total Result
The MDMA Rapid Test Dipstick	Results	Positive	Negative	
	Positive	48	0	48
	Negative	0	62	62
Total Result		48	62	110
Sensitivity: >99.0% (92.6 % ~ 100.0%)		Specificity: 99.0% (94.2% ~ 100.0%)		
Accuracy: >99.0% (96.7% ~ 100.0%)		95% Confidence Intervals		

In order to compare the results of Hana Gene's Ecstasy Rapid test kit, using GC/MS method, 250 clinical specimens (104 positive; 146 negative) were collected, and Hana Gene's kit detected 102 positive and 145 of the specimens negative. The results are shown in the table below:

Method		GC/MS		Total Result
The MDMA Rapid Test Dipstick	Results	Positive	Negative	
	Positive	102	1	103
	Negative	2	145	147
Total Result		104	146	250
Sensitivity: 98.1% (93.2 % ~ 99.7%)		Specificity: 99.3% (96.2% ~ 99.9%)		
Accuracy: 98.8% (96.5% ~ 99.7%)		95% Confidence Intervals		

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Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations of methylenedioxy-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 the cut-off value. This information is summarized in the table below:

Methylenedioxy-methamphetamine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
250	-50%	30	30	0
375	-25%	30	25	5
500	Cut-off	30	14	16
625	+25%	30	4	26
750	+50%	30	0	30
1500	3X	30	0	30

Analytical Features:

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

Compound	Concentration (ng/mL)
3,4-Methylenedioxymethamphetamine HCl (MDMA)	500
3,4-Methylenedioxyamphetamine HCl (MDA)(MDA)	3,000
3,4-Methylenedioxyethyl-amphetamine (MDE)	300

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 methylenedioxy-methamphetamine concentrations with GC/MS method 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.

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Methylenedioxy-methamphetamine 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	8	2	9	1	9	1
625	10	1	9	1	9	1	9
750	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Methylenedioxy-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 methylenedioxy-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 methylenedioxy-methamphetamine concentrations of 250 ng/mL and 750 ng/mL. The adjusted pH of the urine was done in a duplicate rapid ecstasy test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the cross reactivity with positive and negative methylenedioxy-methamphetamine test results, a study was carried out. The following compounds did not show any cross reactivity in the test results with 100 µg/ml ecstasy concentration:

Acetamidophenol Dextromethorphan Meprobamate Procaine Acetophenetidin Diclofenac Methamphetamine Promazine N-Acetylprocainamide Diazepam Methadone Promethazine Acetylsalicylic acid Diflunisal Methoxyphenamine D,L-Propranolol Aminopyrine Digoxin Methylphenidate D-Propoxyphene Amitriptyline Dicyclomine Morphine- D-Pseudoephedrine Amobarbital Diphenhydramine 3-β-D-glucuronide Quinacrine Amoxicillin 5,5 - Diphenylhydantoin Morphine sulfate Quinidine Ampicillin Doxylamine Nalidixic acid Quinine L-Ascorbic acid Ecgonine hydrochloride Naloxone Ranitidine D-Amphetamine Ecgonine methylester Naltrexone Salicylic acid D,L-Amphetamine sulfate (-) -ψ-Ephedrine Naproxen Secobarbital L-Amphetamine [1R,2S](-) Ephedrine Niacinamide Serotonin

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Apomorphine L - Epinephrine Nifedipine (5-Hydroxytyramine) Aspartame Erythromycin Nimesulide Sulfamethazine Atropine β-Estradiol Norcodein Sulindac Benzoic acid Estrone-3-sulfate Norethindrone Sustiva Benzoic acid Ethyl-p-aminobenzoate D-Norpropoxyphene Temazepam Benzoyllecgonine Fenoprofen Noscapine Tetracycline Benzphetamine Furosemide D,L-Octopamine Tetrahydrocortisone, Bilirubin Gentisic acid Oxalic acid 3- Acetate (±) - Brompheniramine Hemoglobin Oxazepam Tetrahydrocortisone Buspiron Hydralazine Oxolinic acid 3-(β-D glucuronide) Caffeine Hydrochlorothiazide Oxycodone Tetrahydrozoline Cannabidiol Hydrocodone Oxymetazoline Thebaine Cannabinol Hydrocortisone Papaverine Theophylline Chloralhydrate O-Hydroxyhippuric acid Penicillin-G Thiamine Chloramphenicol p-Hydroxyamphetamine Pentazocine Trans-2- Chlordiazepoxide p-Hydroxy- hydrochloride phenylcyclopropylamine Chlorothiazide methamphetamine Pentobarbital Thioridazine (±) - Chlorpheniramine 3-Hydroxytyramine Perphenazine Tolbutamide Chlorpromazine Imipramine Phencyclidine Trazodone Chlorquine Iproniazid Phenelzine D,L-Tyrosine Cholesterol (±) - Isoproterenol Phenobarbital Triamterene Clomipramine Isosuprine Phentermine Trifluoperazine Clonidine Ketamine Trans-2-phenyl Trimethoprim Cocaethylene Ketoprofen cyclopropylamine Trimipramine Cocaine hydrochloride Labetalol hydrochloride Tryptamine Codeine Levorphanol L-Phenylephrine D,L-Tryptophan Cortisone Loperamide β-Phenylethylamine Tyramine (-) Cotinine Maprotiline Phenylpropanolamine Uric acid Creatinine Meperidine Prednisolone Verapamil Deoxycorticosterone Mephentermine Prednisone Zomepirac.

References

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