



METHADONE

Rapid Test (MTD) strip

Introduction

The MTD Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Methadone in human urine at a cut-off concentration of 300 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 gas chromatography-mass spectrometry (GC-MS).

Summary

Methadone, sold under the brand name Dolophine, is a strong painkiller and an industrial drug that belongs to the group of opioids and is often used to treat addiction to opiates such as opium, heroin and morphine, as well as to relieve severe pains. This drug relieves pain by reducing the activity of the central nervous system. The half-life of Methadone is about 4 to 7 hours at the beginning of use, and in long-term, continuous use it reaches 24 to 48 hours, which, depending on the metabolism of the person, it may be shorter or longer. Taking Methadone without a doctor's prescription causes very serious complications and consequences for the person in the long run. This drug may even affect the internal organs of the body and lead to serious injuries and death. Other side effects of Methadone use include breathing problems, mental disorders such as depression, oral and dental problems, mood and personality problems, etc. This test uses monoclonal antibodies that selectively detect increased levels of Methadone in urine. The result of the rapid Methadone test is positive when the level of Methadone in the urine exceeds 300 ng/mL.

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Function Method Basis

Rapid Methadone 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 Methadone in the urine is below 300 ng/mL, it does not bind to the binding sites of the antibodies coated with nanoparticles. Consequently, the conjugated antibody will be absorbed by the Methadone on the surface of the nitrocellulose membrane and will leave a colored line in the test area (T). Should the amount be greater than 300 ng/mL, no colored line will appear in the T area, since all the binding sites of the Methadone antibodies are occupied. So, if the presence of Methadone 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 Methadone 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 anti-Methadone monoclonal antibody particles and a combination of conjugated protein-Methadone. 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

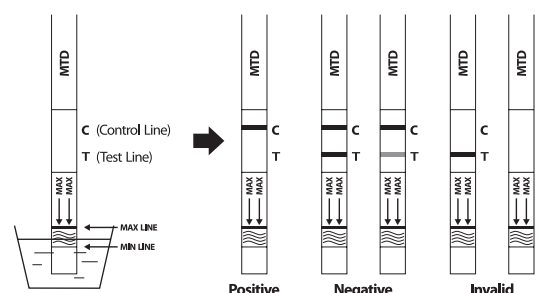
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

• 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 Methadone concentration is below the detectable level of 300 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 Methadone is above the detectable level of 300 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 to confirm the method and the correctness of the test.

Limitations

1. Methadone 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 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 Methadone concentration is less than 300 ng/mL, the result will be negative and if the concentration is greater than 300 ng/mL, the result will be positive. The cut-off value of the test is 300 ng/mL.

Executive Feature

Accuracy: The results obtained from Hana Gene's Methadone Rapid Test strip were compared with those of an available commercial kit. In a study on 210 urine specimens (85 positive and 125 negative), Hana Gene's kit detected 85 of the specimens positive and 122 negative. The results are presented in table below:

Method		other MTD Rapid Test		Total Result
The MTD Rapid Test Dipstick	Results	Positive	Negative	
	Positive	85	3	
	Negative	0	122	
Total Result		85	125	210
% Agreement with this Rapid Test		> 99.9%	> 99.9%	> 99.9%

In order to compare the results of Hana Gene's Cocaine Rapid test kit, using TLC method, 240 clinical specimens (112 positive; 128 negative) were collected, and Hana Gene's kit detected 109 positive and 122 of the specimens negative. The results are shown in the table below:

Method		TLC		Total Result
The MTD Rapid Test Dipstick	Results	Positive	Negative	
	Positive	109	6	
	Negative	3	122	
Total Result		112	128	240
Sensitivity: > 97.3%		Specificity: 95.3%		
Accuracy: 96.2%		95% Confidence Intervals		

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Analytical Sensitivity:

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

0 ng/mL, 150 ng/mL, 225 ng/mL, 300 ng/mL, 375 ng/mL, 450 ng/mL and 900 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:

MTD Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	22	8
300	Cut-off	30	4	26
375	+25%	30	1	29
450	+50%	30	0	30
900	3X	30	0	30

Analytical Features:

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

Compound	Concentration (ng/mL)
Methadone	300
Doxylamine	100,000

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 Methadone concentrations with HPLC 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 300 ng/mL.

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MTD Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
150	10	10	0	10	0	10	0
225	10	10	0	9	1	10	0
375	10	1	9	0	10	1	9
450	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Methadone with concentrations of 150 ng/mL and 450 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain Methadone 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 Methadone concentrations of 150 ng/mL and 450 ng/mL. The adjusted pH of the urine was done in a duplicate rapid 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 manufacturer company producing the antigen and antibody, the following compounds did not show any interference in the test results:

Acetaminophen	Diazepam	Maprotiline	β-Phenylethylamine
Acetophenetidin	Diclofenac	Meperidine	Phenylpropanolamine
N-Acetylprocainamide	Diffunisal	Meprobamate	Prednisolone
Acetylsalicylic acid	Digoxin	Methamphetamine	Prednisone
Aminopyrine	Diphenhydramine	Methoxyphenamine	Procaine
Amitypyline	EDDP	(±) 3,4-Methylenedioxy-	Promazine
Amobarbital	EMDP	amphetamine	Promethazine
Amoxicillin	Ecgonine hydrochloride	(±) 3,4-Methylenedioxy-meth	D,L-Propranolol
Ampicillin	Ecgonine methylester	Amphetamine	D-Propoxyphene
L-Ascorbic acid	(-)-ψ-Ephedrine	Morphine-3-	D-Pseudoephedrine
DL-Amphetamine sulfate	(1R,2S) (-) Ephedrine	β-D glucuronide	Quinacrine
Apomorphine	L - Epinephrine	Morphine Sulfate	Quinidine
Aspartame	Erythromycin	Nalidixic acid	Quinine
Atropine	β-Estradiol	Naloxone	Rantidine
Benzilic acid	Estrone-3-sulfate	Naltrexone	Salicylic acid

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Benzoic acid	Ethyl-p-aminobenzoate	Naproxen	Secobarbital
Benzoylecgonine	Fenoprofen	Niacinamide	Serotonin
Benzphetamine	Furosemide	Nifedipine	Sulfamethazine
Bilirubin	Gentisic acid	Norcodeine	Sulindac
(±) - Brompheniramine	Hemoglobin	Norethindrone	Temazepam
Caffeine	Hydralazine	D-Norpropoxyphene	Tetracycline
Cannabidiol	Hydrochlorothiazide	Noscapine	Tetrahydrocortisone,
Cannabinol	Hydrocodone	D,L-Octopamine	3-Acetate
Chloralhydrate	Hydrocortisone	Oxalic acid	Tetrahydrocortisone
Chloramphenicol	O-Hydroxyhippuric acid	Oxazepam	3-(β-D-glucuronide)
Chlorothiazide	p-Hydroxyamphetamine	Oxolinic acid	Tetrahydrozoline
(±) - Chlorpheniramine	p-Hydroxy-	Oxycodone	Thebaine
Chlorpromazine	methamphetamine	Oxymetazoline	Thiamine
Chlorquine	3-Hydroxytyramine	Papaverine	Thioridazine
Cholesterol	Ibuprofen	Penicillin-G	D,L-Tyrosine
Clomipramine	Imipramine	Pentazocine hydrochloride	Tolbutamide
Clonidine	Iproniazid	Pentobarbital	Triamterene
Cocacethylene	(±) - Isoproterenol	Perphenazine	Trifluoperazine
Cocaine hydrochloride	Isoxsuprine	Phencyclidine	Trimethoprim
Cocaine	Ketamine	Phenelzine	Trimipramine
Cortisone	Ketoprofen	Phenobarbital	Tryptamine
(-) Cotinine	Labetalol	Phentermine	D,L-Tryptophan
Creatinine	Levorphanol	Trans-2-phenyl	Tyramine
Deoxycorticosterone	Loperamide	Cyclopropylamine	Uric acid
Dextromethorphan	Mephentermine	L-Phenylephrine	Verapamil
Zomepirac			

References

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