



MORPHINE

Rapid Test (MOP) strip

Introduction

The MOP Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Morphine 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

Morphine is a strong narcotic causing pain relief by having an effect on the central nervous system. Morphine stops and reduces feelings of pain affecting the user's nervous system. Its consumption causes symptoms such as relaxation, sleepiness, lower body temperature, pain relief, etc. Morphine injection is used to treat severe pain. The injection is subcutaneous, intramuscular, or intravenous, however, the intravenous method is performed only for people who have had a heart attack and are in great pain. The substance is highly addictive and so if the patient has Morphine injections for 2 or 3 times, addiction will develop, leading an individual to a severe physical and psychological need to continue taking it. The symptoms of Morphine addiction and its withdrawal start from around 6 to 8 hours after stopping the use, and reach their highest within 36 to 72 hours, and then during 7 to 10 days they gradually disappear. This test employs monoclonal antibodies for the selective detection of high levels of Morphine in the urine. Should the level be greater than 300 ng/mL, the test result will be positive.

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Function Method Basis

Rapid Morphine 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 Morphine in the urine is below 300 ng/mL, it does not bind to the binding sites of the anti-Morphine antibodies conjugated with gold nanoparticles and binds to the coated antigen in the test area (T) and will leave a colored line there. So, if the presence of Morphine 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 Morphine 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 coated Morphine with anti-Morphine 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 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.

+ **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.

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Contents and Required Supplies

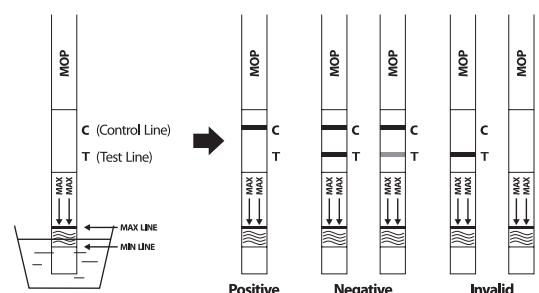
Items found in the kit: + Brochure + Test strip

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 Morphine 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 Morphine 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 in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Morphine 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 spectrophotometry (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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+ **Expected values:** if Morphine 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.

Accuracy: The results obtained from Hana Gene's MOP Rapid Test strip were compared with those of an available commercial kit. In a study on 220 urine specimens (110 positive and 110 negative), Hana Gene's kit detected 110 of the specimens positive and 109 negative. The results are presented in table below:

Method		HANAGENE Result		Total Result
The MOP Rapid Test Dipstick	Results	Positive	Negative	
	Positive	110	1	111
	Negative	0	109	109
Total Result		110	110	220

Sensitivity: > 99% (96.7 % ~ 100%)

Accuracy: 99.55% (97.2% ~ 99.8%)

Specificity: 99.0% (95.0% ~ 99.98%)

95% Confidence Intervals

In order to compare the results of Hana Gene's Morphine Rapid test kit, using TLC method, 280 clinical specimens (136 positive; 144 negative) were collected, and Hana Gene's kit detected 133 positive and 138 of the specimens negative. The results are shown in the table below:

Method		TLC		Total Result
The MOP Rapid Test Dipstick	Results	Positive	Negative	
	Positive	133	6	139
	Negative	3	138	141
Total Result		136	144	280

Sensitivity: > 97.7%

Accuracy: 96.8%

Specificity: 95.8%

95% Confidence Intervals

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

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:

Morphine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Positive	Negative
0	0	30	0	30
150	-50%	30	0	30
225	-25%	30	9	21
300	Cut-off	30	26	4
375	+25%	30	29	1
450	+50%	30	30	0

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

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Codeine	200	Morphine	300
Ethylmorphine	6,000	Norcodeine	6,000
Hydrocodone	50,000	Normorphine	50,000
Hydromorphone	3,000	Oxycodone	30,000
Levorphanol	1,500	Oxymorphone	50,000
6-Monoacetylmorphine	300	Procaine	15,000
Morphine 3-β-D-glucuronide	800	Thebaine	6,000

A panel of coded specimens containing certain Morphine 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 300 ng/mL.

Morphine 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	9	1	8	2	9	1
375	10	0	10	0	10	0	10
450	10	0	10	0	10	0	10

Morphine 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 Morphine concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given Morphine concentrations of 150 ng/mL and 450 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Morphine test whose results show that the pH range does not interfere with the test.

In order to assess the interferences with positive and negative Morphine test results, a study was carried out. The following compounds did not show any interference in the test results with 100 µg/ml Morphine concentration:

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
 -Estradiol Naltrexone Sulfamethazine Benzilic acid Estrone 3-sulfate Naproxen Sulindac
 Benzoic acid Ethyl-paminobenzoate Niacinamide Temazepam Benzoyllecgonine Fenfluramine
 Nifedipine Tetracycline Benzphetamine Fenopropfen Norethindrone Tetrahydrocortisone,
 Bilirubin Furosemide D-Norpropoxyphene 3-Acetate -Brompheniramine Gentisic acid Noscapiene
 Tetrahydrocortisone Caffeine Hemoglobin D,L-Octopamine 3-D glucuronide) Cannabidiol
 Hydralazine Oxalic acid Tetrahydrozoline Chloralhydrate Hydrochlorthiazide Oxazepam
 Thiamine Chloramphenicol Hydrocodone Oxolinic acid Thioridazine Chloridazepoxide
 Hydrocortisone Oxycodone D, L-Tyrosine Chlorothiazide p-Hydroxyamphetamine
 Oxymetazoline Tolbutamide Chlorpheniramine O-Hydroxyhippuric acid Papaverine Trans-2-
 phenyl Chlorpromazine 3-Hydroxytyramine Penicillin-G cyclopropylamine Chlorquine Ibuprofen
 Pentobarbital Triamterene Cholesterol Imipramine Perphenazine Trifluoperazine Clomipramine
 Iproniazide Phenclidine Trimethoprim Clonidine-Isoproterenol Phenelzine Trimipramine
 Coccaethylene Isosuxrine Phenobarbital Tryptamine Cocaine hydrochloride Ketamine
 Phentermine D, L-Tryptophan Codeine Ketoprofen L-Phenylephrine Tyramine Cortisone
 Labetalol ±Phenylethylamine Uric acid (-) Cotinine Levorphanol Phenylpropanolamine
 Verapamil Prednisolone Zomepirac

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