



COCAINE (COC) Rapid Test (COC) strip

Introduction

The COC Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Cocaine 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

Cocaine is a strong central nervous system stimulant and local anesthetic. Potentially, Cocaine brings high energy and restlessness, as well as high sensitivity and spasms when used. Cocaine in large amounts causes fever, breathing problems, and unconsciousness. It is usually consumed by inhalation and intravenous injection. After use in a short period of time, Cocaine is excreted in urine in the form of Benzoylcegonine. Benzoylcegonine is a major metabolite of Cocaine and has a longer biological life than Cocaine (5 to 8 hours) and is usually detectable within 24 to 48 hours after use. COC Rapid Test is a urine screening test to detect increased levels of Cocaine metabolite in the urine. The test result becomes positive when the concentration level of Cocaine metabolite in urine is greater than 300 ng/mL. This screening test is provided by mental health centers and drug abuse treatment service providers.

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Function Method Basis

Rapid Cocaine urine test employs an immunoassay 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 Cocaine in the urine is below 300 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 Cocaine on the surface of the 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 Cocaine antibodies are occupied. So, if the presence of Cocaine 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 Cocaine 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-Cocaine monoclonal antibody particles and a combination of conjugated Protein-Cocaine. 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

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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

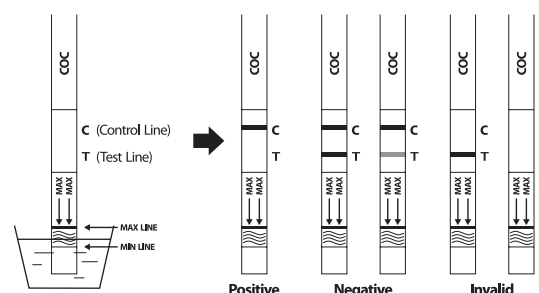
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

- 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 Cocaine 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 Cocaine 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. Cocaine 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 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 Cocaine 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 COC Rapid Test strip were compared with those of an available commercial kit. In a study on 100 urine specimens (40 positive and 60 negative), Hana Gene's kit detected 40 of the specimens positive and 60 negative. The results are presented in table below:

Method		other COC Rapid Test		Total Result
The COC Rapid Test Dipstick	Results	Positive	Negative	
	Positive	40	0	
	Negative	0	60	
Total Result		40	60	100

Sensitivity: > 99% (91.1 % ~ 100%)

Specificity: > 99.0% (94.0% ~ 100.0%)

Accuracy: 99.0% (96.3% ~ 100.0%)

95% Confidence Intervals

In order to compare the results of Hana Gene's Cocaine Rapid test kit, using GC/MS method, 250 clinical specimens (113 positive; 137 negative) were collected, and Hana Gene's kit detected 111 positive and 134 of the specimen's negative. The results are shown in the table below:

Method		GC/MS		Total Result
The COC Rapid Test Dipstick	Results	Positive	Negative	
	Positive	111	3	
	Negative	2	134	
Total Result		113	137	250

Sensitivity: 98.2% (93.7 % ~ 99.7%)

Specificity: 97.8% (93.7% ~ 99.5%)

Accuracy: 98.0% (95.3% ~ 99.3%)

95% Confidence Intervals

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Analytical Sensitivity:

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

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:

Benzoyllecgonine 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	26	4
300	Cut-off	30	13	17
375	+25%	30	3	27
450	+50%	30	0	30
900	3X	30	0	30

Analytical Features:

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

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Benzoyllecgonine	300	Cocaethylene	12,500
Cocaine HCl	200	Ecgonine HCl	30,000

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 benzoyllecgonine 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.

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Benzoyllecgonine 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	9	2	9	1
375	10	1	9	2	9	2	9
450	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Benzoyllecgonine 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 benzoyllecgonine 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 benzoyllecgonine concentrations of 150 ng/mL and 450 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Cocaine test whose results show that the pH range does not interfere with the test.

Cross Reactivity

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

0.3	Diazepam	Methadone	Prednisone
Acetophenetidin	Diclofenac	Methoxyphenamine	Procaine
N-Acetylprocainamide	Diffunisal	(±)-3,4-Methylenedioxy-	Promazine
Acetylsalicylic acid	Digoxin	amphetamine	Promethazine
Aminopyrine	Diphenhydramine	(±)-3,4-Methylenedioxy-	D,L-Propranolol
Amitypyline	Doxylamine	methamphetamine	D-Propoxyphene
Amobarbital	Ecgonine methylester	Morphine-3-β-D	D-Pseudoephedrine
Amoxicillin	(-)-ψ-Ephedrine	glucuronide	Quinidine
Ampicillin	Erythromycin	Morphine Sulfate	Quinine
L-Ascorbic acid	β-Estradiol	Nalidixic acid	Ranitidine
D,L-Amphetamine sulfate	Estrone-3-sulfate	Naloxone	Salicylic acid
Apomorphine	Ethyl-p-aminobenzoate	Naltrexone	Secobarbital
Aspartame	Fenoprofen	Naproxen	Serotonin
Atropine	Furosemide	Niacinamide	Sulfamethazine

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Benzilic acid	Gentisic acid	Nifedipine	Sulindac
Benzoic acid	Hemoglobin	Norcodeine	Temazepam
Benzphetamine	Hydralazine	Norethindrone	Tetracycline
Bilirubin	Hydrochlorothiazide	D-Norpropoxyphene	Tetrahydrocortisone,
(±)-Brompheniramine	Hydrocodone	Noscapine	3-Acetate
Caffeine	Hydrocortisone	D,L-Octopamine	Tetrahydrocortisone
Cannabidiol	O-Hydroxyhippuric acid	Oxalic acid	3-(β-D glucuronide)
Cannabinol	p-Hydroxy-	Oxazepam	Tetrahydrozoline
Chloralhydrate	methamphetamine	Oxolinic acid	Thebaine
Chloramphenicol	3-Hydroxytyramine	Oxycodone	Thiamine
Chlordiazepoxide	Ibuprofen	Oxymetazoline	Thioridazine
Chlorothiazide	Imipramine	Papaverine	D,L-Tyrosine
(±)-Chlorpheniramine	Iproniazid	Penicillin-G	Tolbutamide
Chlorpromazine	(±)-Isoproterenol	Pentobarbital	Triamterene
Chlorquine	Isosuprine	Perphenazine	Trifluoperazine
Cholesterol	Ketamine	Phencyclidine	Trimethoprim
Clomipramine	Ketoprofen	Phenelzine	Trimipramine
Clonidine	Labetalol	Phenobarbital	Tryptamine
Codeine	Levorphanol	Phentermine	D,L-Tryptophan
Cortisone	Loperamide	L-Phenylephrine	Tyramine
(-) Cotinine	Maprotiline	β-Phenylethylamine	Uric acid
Crotinine	Meperidine	Phenylpropanolamine	Verapamil
Deoxycorticosterone	Meprobamate	Prednisolone	Zomepirac
Dextromethorphan			

References

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