COT Rapid Test Cassette (Urine)
Package Insert

REF DCT-102 | English

A rapid test for the qualitative detection of Cotinine (nicotine metabolite) in human urine.
For determination of smoking status only. Not intended for medical diagnostic use.

【INTENDED USE】
The COT Rapid Test Cassette (Urine) is a rapid chromatographic immunoassay for the detection of Cotinine in human urine at a cut-off concentration of 200 ng/mL. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.
This assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography and mass spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

【SUMMARY】
Cotinine is the first-stage metabolite of nicotine, a toxic alkaloid that produces stimulation of the autonomic ganglia and central nervous system when in humans. Nicotine is a drug to which virtually every member of a tobacco-smoking society is exposed whether through direct contact or second-hand inhalation. In addition to tobacco, nicotine is also commercially available as the active ingredient in smoking replacement therapies such as nicotine gum, transdermal patches and nasal sprays.
In a 24-hour urine, approximately 5% of a nicotine dose is excreted as unchanged drug with 10% as cotinine and 35% as hydroxycotinine; the concentrations of other metabolites are believed to account for less than 5%.[1] While cotinine is thought to be an inactive metabolite, it's elimination profile is more stable than that of nicotine which is largely urine pH dependent. As a result, cotinine is considered a good biological marker for determining nicotine use. The plasma half-life of nicotine is approximately 60 minutes following inhalation or parenteral administration.[2] Nicotine and cotinine are rapidly eliminated by the kidney; the window of detection for cotinine in urine at a cutoff level of 200 ng/mL is expected to be up to 2-3 days after nicotine use.
The COT Rapid Test Cassette (Urine) is a rapid urine screening test that can be performed without the use of an instrument. The test utilizes a monoclonal antibody to selectively detect elevated levels of Cotinine in urine. The COT Rapid Test Cassette (Urine) yields a positive result when the Cotinine in urine exceeds 200 ng/mL.

【PRINCIPLE】
The COT Rapid Test Cassette (Urine) is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.
During testing, a urine specimen migrates upward by capillary action. Cotinine, if present in the urine specimen below 200 ng/mL, will not saturate the binding sites of antibody coated particles in the test . The antibody coated particles will then be captured by immobilized Cotinine conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Cotinine level exceeds 200 ng/mL because it will saturate all the binding sites of anti-Cotinine antibodies.
A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that that proper volume of specimen has been added and membrane wicking has occurred.

【REAGENTS】
The test contains mouse monoclonal anti-Cotinine antibody-coupled particles and Cotinine-protein conjugate. A goat antibody is employed in the control line system.

【PRECAUTIONS】
- For medical and other professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test should be discarded according to local regulations.

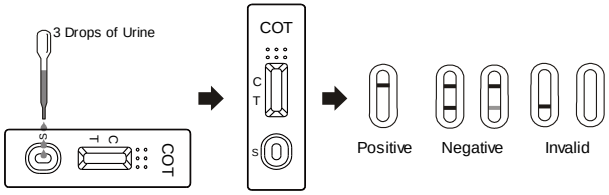
【STORAGE AND STABILITY】
Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

【SPECIMEN COLLECTION AND PREPARATION】
Urine Assay
The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be centrifuged, filtered, or allowed settle to obtain a clear supernatant for testing.
Specimen Storage
Urine specimens may be stored at 2-8°C for up to 48 hours prior to assay. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

【MATERIALS】
Materials Provided
- Test cassettes
- Droppers
- Package insert

Materials Required But Not Provided
- Specimen collection container
- Timer

【DIRECTIONS FOR USE】
Allow test, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.
1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test cassette on a clean and level surface. Hold the dropper vertically and **transfer 3 full drops of urine** (approx. 120 μL) to the specimen well (S) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S). See the illustration below.



3. Wait for the colored line(s) to appear. The result should be **read at 5 minutes.** It is important that the background is clear before the result is read. Do not interpret the result after 10 minutes.

【INTERPRETATION OF RESULTS】
(Please refer to the illustration above)
NEGATIVE:* Two lines appear. One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the Cotinine concentration is below the detectable level (200 ng/mL).
***NOTE:** The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.
POSITIVE: One colored line appears in the control line region (C). No line appears in the test line region (T). This positive result indicates that the Cotinine concentration exceeds the detectable level (200 ng/mL).
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test. If the problem persists, discontinue using the lot immediately and contact your local distributor.

【QUALITY CONTROL】
A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

【LIMITATIONS】
1. The COT Rapid Test Cassette (Urine) provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1,2]
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. Test does not distinguish between drugs of abuse and certain medications.

【EXPECTED VALUES】
This negative result indicates that the Cotinine concentration is below the detectable level of 200ng/ml. Positive result means the concentration of Cotinine is above the level of 200ng/ml. The COT Rapid Test Cassette has a sensitivity of 200ng/ml.

【PERFORMANCE CHARACTERISTICS】
Accuracy
A comparison was conducted using the COT Rapid Test Cassette (Urine) and GC/MS. The following results were tabulated:

Method		GC/MS		Total Results
COT Rapid Test Cassette	Results	Positive	Negative	
	Positive	88	4	92
	Negative	3	155	158
Total Results		91	159	250
% Agreement		96.7%	97.5%	97.2%

Analytical Sensitivity
A drug-free urine pool was spiked with Cotinine at the following concentrations: 0 ng/mL, 100 ng/mL, 150 ng/mL, 200 ng/mL, 250 ng/mL, 300 ng/mL and 600 ng/mL. The results demonstrate > 99% accuracy at +50% above and 50% below the cut-off concentration. The data are summarized below:

Cotinine Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
100	-50%	30	30	0
150	-25%	30	27	3
200	Cut-off	30	15	15
250	+25%	30	4	26
300	+50%	30	0	30
600	+300%	30	0	30

Analytical Specificity
The following table lists compounds that are positively detected in urine by the COT Rapid Test Cassette (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
(-)-Cotinine	200
(-)-Nicotine	5,000

Precision
A study was conducted at three hospitals by laypersons using three different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing, according to GC/MS, no Cotinine, 25% Cotinine above and below the cut-off, and 50% Cotinine above and below the 200 ng/mL cut-off was provided to each site. The results are given below:

Cotinine Concentration (ng/mL)	n per site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
100	10	10	0	10	0	10	0
150	10	9	1	9	1	9	1
250	10	1	9	1	9	2	8
300	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity
Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 100 ng/mL and 300 ng/mL of Cotinine. The COT Rapid Test Cassette (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

Effect of Urinary pH
The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with Cotinine to 100 ng/mL and 300 ng/mL. The spiked, pH-adjusted urine was tested with the COT Rapid Test Cassette (Urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

Cross-Reactivity
A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or Cotinine positive urine. The following compounds show no cross-reactivity when tested with the COT Rapid Test Cassette (Urine) at a concentration of 100 μg/mL.

Non Cross-Reacting Compounds

4-Acetamidophenol	4-Dimethylaminoantipyrine	Lithium carbonate	Phentermine
Acetone	Diphenhydramine	Loperamide	trans-2-Phenyl cyclopropylamine
Acetophenetidin	5,5-Diphenylhydantoin	Maprotiline	l-Phenylephrine
Acetylsalicylic acid	Disopyramide	Meperidine	β-Phenylethylamine
N-Acetylprocainamide	Doxylamine	Mephentermine	Phenylpropanolamine (d,l-norephedrine)
Albumin	Ecgonine	Meprobamate	(±) Phenylpropanolamine
Aminopyrine	Ecgonine methylester	Methadone	Prednisolone
Amitriptyline	EDDP	d-Methamphetamine	Prednisone
Amobarbital	Efavirenz (Sustiva)	l-Methamphetamine	5β-Pregnane-3α, 17α, 21-triol
Amoxapine	EMDP	Methaqualone	Procaine
Amoxicillin	Ephedrine	Methoxyphenamine	Promazine
l-Amphetamine	l-Ephedrine	(-) 3,4-Methylenedioxy-amphetamine (MDA)	Promethazine
Ampicillin	(±)-Epinephrine	(+) 3,4 Methylendioxy-methamphetamine (MDMA)	d,l-Propanolol
Apomorphine	l-Epinephrine	Methylphenidate	d-Propoxyphene
l-Ascorbic acid	Erythromycin	Methyprylon	d-Pseudoephedrine
Aspartame	β-Estradiol	Methaqualone	Quinacrine
Atropine	Estrone-3-sulfate	Metoprolol	Quinidine
Benzilic acid	Ethanol (Ethyl alcohol)	Morphine sulfate	Quinine
Benzoic acid	Ethyl-p-aminobenzoate	Morphine-3-β-D-glucuronide	Ranitidine
Benzoylecgonine	Etodolac	Nalidixic acid	Riboflavin
Benzphetamine	Famprofazone	Nalorphine	Salicylic acid
Bilirubin	Fenfluramine	Naloxone	Secobarbital
(±)-Bromopheniramine	Fenoprofen	Naltrexone	Serotonin (5-hydroxytryptamine)
Buspirone	Fentanyl	Methyprylon	Sodium chloride
Caffeine	Fluoxetine	Metoprolol	Sulfamethazine
Cannabidiol	Furosemide	Nimesulide	Sulindac
Cannabinol	Gentisic acid	Norcodein	Temazepam
Chloral hydrate	d (+) Glucose	Morphine sulfate	Tetracycline
Chloramphenicol	Guaiacol glyceryl ether	α-Naphthaleneacetic acid	Tetrahydrocortisone, 3-acetate
Chlordiazepoxide	Guaiacol glyceryl ether carbamate	Norethindrone	Tetrahydrozoline
Chloroquine	Hemoglobin	Normorphine	Thebaine
Chlorothiazide	Hydralazine	d-Norpropoxyphene	Theophylline
(+)-Chlorpheniramine	Hydrochlorothiazide	Noscapine	Thiamine
(±)-Chlorpheniramine	Hydrocodone	d,l-Octopamine	Thioridazine (chlorpromazine)
Chlorpromazine	Hydrocortisone	Orphenadrine	l-Thyroxine
Chlorprothixene	Hydromorphone	Oxalic acid	Tolbutamide
Cholesterol	p-Hydroxyamphetamine	Oxazepam	cis-Tramadol
Cimetidine	o-Hydroxyhippuric acid	Oxolinic acid	Trazodone
Clomipramine	p-Hydroxymethamphetamine	Oxycodone	Triamterene
Clonidine	p-Hydroxynorephedrine	Oxymetazoline	Trifluoperazine
Cocaine	Hydroxyzine	Oxymorphone	Trimethobenzamide
Codeine	3-Hydroxytyramine	Papaverine	Trimethoprim
Cortisone	Ibuprofen	Pemoline	Trimipramine
Creatinine	Imipramine	Penicillin-G	Tryptamine
Cyclobarbital	Iproniazid	Pentazocine	d,l-Tryptophan
Cyclobenzaprine	(-)-Isoproterenol	Pentobarbital	Tyramine
Deoxycorticosterone	Isoxsuprine	Perphenazine	d,l-Tyrosine
(-) Deoxyephedrine	Kanamycin	Phencyclidine	Uric acid
R (-) Deprenyl	Ketamine	Phenelzine	Verapamil
Dextromethorphan	Ketoprofen	Pheniramine	Zomepirac
Diazepam	Labetalol	Phenobarbital (hexachlorocyclohexane)	
Diclofenac	Levorphanol	Phenothiazine	
Dicyclomine	Lidocaine		
Diflunisal	Lindane		
Digoxin			

【BIBLIOGRAPHY】

1. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 6th Edition. Biomedical Publications, Foster City, CA. 2002; 744-747
2. Hardman JG, Limbird LE. Goodman and Gilman's: The Pharmacological Basis for Therapeutics. 10th Edition. McGraw Hill Medical Publishing, 2001; 208-209.

Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For in vitro diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged				

Hangzhou AllTest Biotech Co., Ltd.
#550, Yinhai Street
Hangzhou Economic & Technological Development Area
Hangzhou - 310018, P. R. China
www.alltests.com.cn

EC REP
MedNet GmbH
Borkstrasse 10
48163 Muenster
Germany

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