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COT

One Step
Cotinine Test Device
Package Insert

A rapid, one step test for the qualitative detection of Cotinine (nicotine metabolite) in human urine.

For professional in vitro diagnostic use only.

INTENDED USE

The COT One Step Cotinine Test Device (Urine) is a lateral flow 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. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated. Should a more specific chemical method be requested, gas chromatography/mass spectrometry (GC/MS), is the preferred confirmatory method.

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 One Step Cotinine Test Device (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 One Step Cotinine Test Device (Urine) yields a positive result when the Cotinine in urine exceeds 200 ng/mL.

PRINCIPLE

The COT One Step Cotinine Test Device (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 device. 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 device contains mouse monoclonal anti-Cotinine antibody-coupled particles and Cotinine-protein conjugate. A goat antibody is employed in the control line system.

PRECAUTIONS

- The device is for professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test device 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 device 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 device is stable through the expiration date printed on the sealed pouch. The test device 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 devices
- Droppers
- Package insert

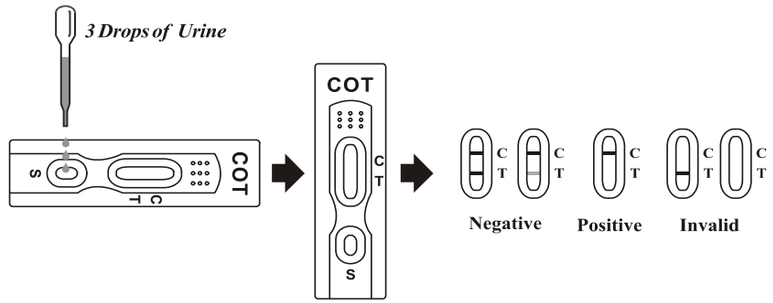
Materials Required But Not Provided

- Specimen collection container
- Timer

DIRECTIONS FOR USE

Allow test device, urine specimen to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Hold the dropper vertically and **transfer 3 full drops of urine** (approx. 100 µL) to the specimen well (S) of the test device, 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 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 device. 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 procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

LIMITATION

1. This assay provides only a preliminary analytical test result. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated. Should a more specific chemical method be requested, 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 only that the presence of Cotinine is above the cut-off concentration. It does not indicate or measure level of consumption.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the COT One Step Cotinine Test Device (Urine) and a leading commercially available COT rapid test. Testing was performed on 300 clinical specimens collected from smoking and non-smoking volunteers. The following results were tabulated:

Method		Other COT Rapid Test		Total
COT One Step Test Device	Results	Positive	Negative	Results
	Positive	103	12	115
	Negative	0	185	185
Total Results		103	197	300
% Agreement		>99%	94%	96%

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 400 ng/mL. The result demonstrates > 99% accuracy at 100% 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	90	90	0
100	-50%	90	90	0
150	-25%	90	90	0
200	Cut-off	90	63	27
250	+25%	90	40	50
300	+50%	90	16	74
400	+100%	90	0	90

Analytical Specificity

The following table lists compounds that are positively detected in urine by the COT One Step Cotinine Test Device (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
(-)-Cotinine	200
(-)-Nicotine	6,250

Precision

A study was conducted by trained operators using 2 different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing no Cotinine, 50% Cotinine below cut-off level and 100% Cotinine above the 200 ng/mL cutoff level were used. The following results were tabulated:

Cotinine Concentration (ng/mL)	n per lot	Lot A		Lot B	
		-	+	-	+
0	30	30	0	30	0
100	30	30	0	30	0
400	30	0	30	0	30

Effect of Urinary Specific Gravity

Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 100 ng/mL and 400 ng/mL of Cotinine. The COT One Step Cotinine Test Device (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 400 ng/mL. The spiked, pH-adjusted urine was tested with the COT One Step Cotinine Test Device (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 One Step Cotinine Test Device (Urine) at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

4-Acetaminophenol	Acetone	Acetophenetidin	Acetylsalicyclic acid
Albumin	Aminopyrine	Amitryptyline	Amobarbital
Amoxapine	Amoxicillin	l-Amphetamine	Ampicillin
Apomorphine	Aspartame	Atropine	Benzilic acid
Benzoic acid	Benzoylecgonine	Benzphetaminne	Bilirubin
Brompheniramine	Buspirone	Caffeine	Cannabidiol
Cannabinol	Chloral Hydrate	Chloramphenicol	Chlordiazepoxide
Chloroquine	(+)-Chlorpheniramine	(±)Chlorpheniramine	Chlorpromazine
Chlorprothixene	Cholestrol	Cimetidine	Clomipramine
Clonidine	Cocaine	Codeine	Cortisone
Creatinine	Cyclobarbital	Cyclobenzaprine	Deoxycorticosterone
(-) Deoxyephedrine	R(-) Deprenyl	Dextromethorphan	Diazepam
Diclofenac	Digoxin	4-Dimethylaminoantipyrine	Diphenhydramine
5,5-Diphenylhydantoin	Disopyramide	Doxylamine	Ecgonine
Ecgonine Methylester	EDDP	Efavirenz (Sustiva)	EMDP
Ephedrine	(1r,2s)-(-) Ephedrine	(-)ψ-Ephedrine	(±)Epinephrine
Erythromycin	B-Estradiol	Estrone 3-sulfate	Ethanol
Ethyl-p-aminobenzoate	Etodolac	Famprofazone	Fenfluramine
Fenoprofen	Fentanyl	Fluoxetine	Furosemide
Gentisic acid	d (+) Glucose	Guaiacol Glyceryl Ether	Hemoglobin
Hydralazine	Hydrochlorothiazide	Hydrocodone	Hydrocortisone
Hydromorphone	p-Hydroxyamphetamine	o-Hydroxyhippuric acid	p-Hydroxymethamphetamine
p-Hydroxynorephedrine	Hydroxyzine	3-Hydroxytyramine	Ibuprofen
Imipramine	Iproniazid	(-)Isoproterenol	Isoxsuprine
Kanamycin	Ketamine	Ketoprofen	Labetalol
l-Ascorbic acid	l-Ephedrine	l-Epinephrine	Levorphanol
Lidocaine	Lindane	Lithum Carbonate	Loperamide
Maprotiline	Meperidine	Mephentermine	Meprobamate
Methadone	d-Methamphetamine	l-Methamphetamine	Methaqualone
Methoxyphenamine	MDA*	MDMA**	Methylphenidate
Methyprylon	Metoprolol	Morphine Sulfate	Morphine 3-β-d-glucuronide
Nalidixic acid	Nalorphine	Naloxone	Naltrexone
Nimesulide	Norcodeine	a-Naphthaleneactetic acid	Norethindrone
Normorphine	d-Norpropoxyphene	Noscapine	d,l-Octopamine
Orphenadrine	Oxalic acid	Oxazepam	Oxolinic acid
Oxycodone	Oxymetazoline	Oxymorphone	Papaverine
Pemoline	Penicillin-G	Pentazocine	Pentobarbital
Perphenazine	Phencyclidine	Phenelzine	Pheniramine
Phenobarbital	Phenothiazine	Phentermine	Trans-2-phenylcyclopropylamine
l-Phenylephrine	B-Phenylethylamine	d,l Norephedrine	(±)Phenylpropanolamine
Prednisolone	Prednisone	Procaine	Promazine
Promethazine	d,l-Propanolol	d-Propoxyphene	d-Pseudoephedrine
Quinacrine	Quinidine	Quinine	Ranitidine
Riboflavin	Salicylic acid	Secobarbital	Serotonin
Sodium Chloride	Sulfamethazine	Sulindac	Temazepam
Tetracycline	Tetrahydrocortisone	3-acetate Tetrahydrozoline	Thebaine
Theophylline	Thiamine	Thioridazine	l-Thyroxine
Tolbutamine	Cis-Tramadol	Trazodone	Trimterene
Trifluoperazine	Trimethobenzamide	Trimethoprim	Trimipramine
Tryptamine	d,l-Tryptophan	Tyramine	d,l-Tyrosine
Uric Acid	Verapmil	Zomepirac	

*MDA= 3,4-Methylenedioxyamphetamine **MDMA = 3,4-Methylenedioxymethamphetamine

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