

**A rapid test for the qualitative detection of Cotinine (nicotine metabolite) in human urine.  
For medical and other professional in vitro diagnostic use only.****INTENDED USE**

The **DrugControl COT Test** is a rapid chromatographic immunoassay for the detection of Cotinine (nicotine metabolite) in human urine at the cut-off concentration of 200ng/ml. The following table lists compounds that are positively detected in urine by the **DrugControl COT Test** at 5 minutes

TEST DEVICE	CALIBRATOR / related compounds	CUT-OFF LIMIT VALUE [ng / ml]
COT 200	(-)-Cotinine	200
	(-)-Nicotine	5,000

This assay provides only a qualitative, preliminary, analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/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%.<sup>1</sup> While cotinine is thought to be an inactive metabolite, its 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.<sup>3</sup> 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 **DrugControl COT Test** 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 **DrugControl COT Test** yields a positive result when the Cotinine in urine exceeds 200 ng/mL.

**TEST PRINCIPLE**

The **DrugControl COT Test** 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 device should remain in the sealed pouch until use.
- Do not use the test if the foil pouch is damaged
- Do not moisten nitrocellulose membrane with urine samples.
- Read the entire procedure carefully prior testing.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Humidity and temperature can adversely affect results.
- The used test device should be discarded according to federal state and local regulations.
- Do not reuse tests
- Avoid cross-contamination of urine samples by using a new specimen collection container for each urine sample.
- Do not eat, drink or smoke in the area where the specimens and kits are handled.

**STORAGE AND STABILITY**

Store as packaged 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. Tests should be kept out of direct sunlight.

- 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 particles should be centrifuged, filtered, or allowed to settle to obtain clear specimen for testing.

### Specimen Storage

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For long-term storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

### MATERIALS PROVIDED

- Test in pouch
- Disposable sample dropper (in pouch)
- Package insert

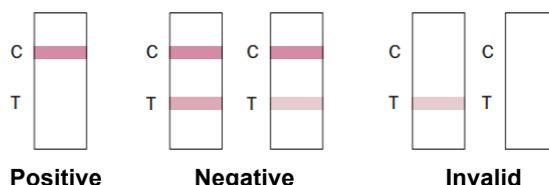
### MATERIALS REQUIRED, BUT NOT PROVIDED

- Specimen collection container
- Timer
- Positive and negative controls

### DIRECTIONS FOR USE

1. Allow the test, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.
2. Bring the pouch to room temperature before opening it.
3. Remove the Test from the sealed pouch and use it within one hour.
4. Place the test cassette on a clean and level surface.
5. Hold the dropper vertically and transfer 3 full drops of urine to the specimen well (S) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S).
6. Read results at 5 minutes. Do not interpret the result after 10 minutes.

### INTERPRETATION OF RESULTS



- Positive:** One color line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the COT concentration is above the detectable cut-off level. (substances & cut-off concentrations see table on page 1).
- Negative:\*** Two lines appear. One color line should be in the control region (C), and another apparent color line should be in the test region (T). This negative result indicates that the COT concentration is below the detectable cut-off level.
- 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 with a new test. If the problem persists, discontinue using the test immediately and contact your local distributor.
- \* Note:** The shade of red in the test line region (T) may vary, but it should be considered negative whenever there is even a faint pink line.

### QUALITY CONTROL

A procedural control is included in the test. A color line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this test; however it is recommended that positive and negative controls be tested as good laboratory testing practices to confirm the test procedure and to verify proper test performance.

### LIMITATIONS

- The **DrugControl COT Test** provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrophotometry (GC/MS) is the preferred confirmatory method<sup>1,2</sup>.
- 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.
- A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
- 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.
- It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
- This test does not distinguish between drugs of abuse and certain medications.
- The **DrugControl COT Test** is intended for use with human urine specimens only.

### 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 **DrugControl COT Test** has a sensitivity of 200ng/ml.

### PERFORMANCE CHARACTERISTICS

#### Accuracy

A comparison was conducted using the **DrugControl COT Test** and GC/MS. The following results were tabulated:

COT		GC/MS		Total Results
		Positive	Negative	
DrugControl COT Test	Positive	88	4	92
	Negative	3	155	158
Total Results		91	159	250
% Agreement with this Test		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:

COT 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	3X	30	0	30

**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:

COT 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 DrugControl COT Test 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 DrugControl COT Test 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 DrugControl COT Test at a concentration of 100 µg/mL.

**Non Cross-Reacting Compounds**

4-Acetamidophenol	Cyclobenzaprine	Hydrocodone	Nalidixic acid	Procaine
Acetone	Deoxycorticosterone	Hydrocortisone	Nalorphine	Promazine
Acetophenetidin	(-) Deoxyephedrine	Hydromorphone	Naloxone	Promethazine
Acetylsalicylic acid	R (-) Deprenyl	p-Hydroxyamphetamine	Naltrexone	d,l-Propranolol
N-Acetylprocainamide	Dextromethorphan	o-Hydroxyhippuric acid	Methpyrlylon	d-Propoxyphene
Albumin	Diazepam	p-Hydroxymethamphetamine	Metoprolol	d-Pseudoephedrine
Aminopyrine	Diclofenac	p-Hydroxynorephedrine	Nimesulide	Quinacrine
Amitriptyline	Dicyclomine	Hydroxyzine	Norcodein	Quinidine
Amobarbital	Diflunisal	3-Hydroxytyramine	Morphine sulfate	Quinine
Amoxapine	Digoxin	Ibuprofen	α-Naphthaleneacetic acid	Ranitidine
Amoxicillin	4-Dimethylaminoantipyrine	Imipramine	Norethindrone	Riboflavin
l-Amphetamine	Diphenhydramine	Iproniazid	Normorphine	Salicylic acid
Ampicillin	5,5-Diphenylhydantoin	(-) Isoproterenol	d-Norpropoxyphene	Secobarbital
Apomorphine	Disopyramide	Isoxsuprine	Noscapine	Serotonin
l-Ascorbic acid	Doxylamine	Kanamycin	d,l-Octopamine	(5-hydroxytryptamine)
Aspartame	Ecgonine	Ketamine	Orphenadrine	Sodium chloride
Atropine	Ecgonine methylester	Ketoprofen	Oxalic acid	Sulfamethazine
Benzilic acid	EDDP	Labetalol	Oxazepam	Sulindac
Benzoic acid	Efaverenz (Sustiva)	Levorphanol	Oxolinic acid	Temazepam
Benzoylecgonine	EMDP	Lidocaine	Oxycodone	Tetracycline
Benzphetamine	Ephedrine	Lindane	Oxymetazoline	Tetrahydrocortisone,
Bilirubin	(±)-Brompheniramine	(hexachlorocyclohexane)	Papaverine	3-acetate
Buspirone	(±)-Epinephrine	Lithium carbonate	Pemoline	Tetrahydrozoline
Caffeine	l-Epinephrine	Loperamide	Penicillin-G	Thebaine
Cannabidiol	Erythromycin	Maprotiline	Pentazocine	Theophylline
Cannabinol	β-Estradiol	Meperidine	Pentobarbital	Thiamine
Chloral hydrate	Estrone-3-sulfate	Mephentermine	Phencyclidine	Thioridazine
Chloramphenicol	Ethanol (Ethyl alcohol)	Meprobamate	Phenelzine	(chlorpromazine)
Chlordiazepoxide	Ethyl-p-aminobenzoate	Methodone		l-Thyroxine
				Tolbutamide

Chloroquine	Etodolac	d-Methamphetamine	Pheniramine	cis-Tramadol
Chlorothiazide	Famprofazone	l-Methamphetamine	Phenobarbital	Trazodone
(+)-Chlorpheniramine	Fenfluramine	Methaqualone	Phenothiazine	Triamterene
(±)-Chlorpheniramine	Fenpropfen	Methoxyphenamine	Phentermine	Trifluoperazine
Chlorpromazine	Fentanyl	(-) 3,4-Methylenedioxy- amphetamine (MDA)	trans-2-Phenyl cyclopropylamine	Trimethobenzamide
Chlorprothixene	Fluoxetine	(+) 3,4 Methylenedioxy- methamphetamine (MDMA)	l-Phenylephrine	Trimethoprim
Cholesterol	Furosemide	Methylphenidate	β-Phenylethylamine	Trimipramine
Cimetidine	Gentisic acid	Methpyrlyon	Phenylpropanolamine (d,l-norephedrine)	Tryptamine
Clomipramine	d (+) Glucose	Methaqualone	(±) Phenylpropanolamine	d,l-Tryptophan
Clonidine	Guaiacol glyceryl ether	Metoprolol	Prednisolone	Tyramine
Cocaine	Guaiacol glyceryl ether carbamate	Morphine sulfate	Prednisone	d,l-Tyrosine
Codeine	Hemoglobin	Morphine- 3-β-D-glucuronide	5β-Pregnane-3α, 17α, 21-triol	Uric acid
Cortisone	Hydralazine			Verapamil
Creatinine	Hydrochlorothiazide			Zomepirac
Cyclobarbital				

### LIMITATIONS

It is impossible to check any and all - other than those drugs mentioned in the product insert - for cross-reactivity or any other influences to the to be detected drug of abuse ( DOA ).

If the patient takes a „cocktail“ of several different drugs or medication cannot be excluded that a non-reproducible cross-reaction can falsified the test result.

### BIBLIOGRAPHY

1. Hardman JG, Limbird LE. Goodman and Gilman's: The Pharmacological Basis for Therapeutics. 10th Edition. McGraw Hill Medical Publishing, 2001; 208-209.
2. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488

 Manufacturer	 Contents sufficient for <n> tests
 For in vitro diagnostic use only	 Lot. no.
 For single use only	 Expiration date
 Read instructions for use	 Store at
 Keep away from direct sunlight	 Ordering number
 Keep dry	

**This operating manual conforms to the latest technology / revision. Subject to change without prior notice!**



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November 2014-AL  
/A NB