CotinineTest Device(Urine)

Specimens: Urine

For professional invitro diagnostic use only.

INTENDED USE

The Cotinine Test Device (Urine) is a rapid visual immunoassay for the qualitative,presumptive detection of Cotinine in human urine specimens at the cut-off concentrations listed below:

Parameter	Calibrator	Cut-off(ng/mL)
COT (Cotinine)	(-)-Cotinine	200

INTRODUCTION

Cotinine is the first-stage metabolite of nicotine, a toxic alkaloid that produces stimulation of theautonomic ganglia and central nervous system when in humans. Nicotine is a drug to which virtuallyevery member of a tobacco-smoking society is exposed whether through direct contact or second-handinhalation.In addition to tobacco, nicotine is also commercially available as the active ingredient insmokingreplacementtherapiessuchasnicotinegum,transdermalpatchesandnasalsprays. Ina24-hoururine,approximately5%ofanicotinedoseisexcretedasunchangeddrugwith10%ascotinineand35%ashydroxycotinine;theconcentrationsofothermetabolitesarebelievedtoaccountforlessthan5%.While cotinine is thought to be an inactive metabolite, it's elimination profile is more stable thanthatofnicotinewhichislargelyurinepHdependent.Asaresult,cotinineisconsideredagoodbiologicalmarkerfordeterminingnicotineuse.Theplasmahalf-lifeofnicotineisapproximately60minutes

following inhalation or parenteral administration. Nicotine and cotinine are rapidly eliminated by thekidney; the window of detection for cotinine in urine at a cutoff level of 200 ng/mLis expected to be upto 2-3 days after nicotine use.

PRINCIPLE

The COT Cotinine Test Device (Urine) detects Cotinine through visual interpretation of colordevelopment on the strip. Drug conjugates are immobilized on the test region of the membrane. Duringtesting, the specimen reacts with antibodies conjugated to colored particles and precoated on the samplepad.Themixturethenmigratesthroughthemembranebycapillaryaction,andinteractswithreagentsonthe membrane. If there are insufficient drug molecules in the specimen, the antibody-colored particleconjugate will bind to the drug conjugates, forming a colored band at the test region of the membrane.Therefore, a colored band appears in the test region when the urine is negative for the drug. If drugmolecules are present in the urine above the cut-off concentration of the test, they compete with theimmobilized drug conjugate on the test region for limited antibody binding sites. This will preventattachment of the antibody-colored particle conjugate to the test region. Therefore, the absence of acoloredband atthetest regionindicatesapositiveresult.Theappearanceofacoloredband atthecontrol

region serves as a procedural control, indicatingthat the proper volume of specimen has been added andmembrane wicking has occurred.

REAGENTS

Each test consists of a reagent strip. The amount of each antigen and/or antibody coated on the strip isless than 0.001 mg for antigen conjugates and goat anti-rabbit IgG antibodies, and less than 0.0015 mgfor antibody components.

The control zone of each test contains goat anti-rabbit IgG antibody. The test zone of each test containsdrug-bovineproteinantigenconjugate,andtheconjugatepadofeachtest containsmonoclonalanti-drugantibody and rabbit antibody-colored particle complex.

MATERIALS

MaterialsProvided

- IndividuallypouchedTestDevices
- Package insert
- Disposablepipettes
-

MaterialsRequiredbutNot provided

- Positiveandnegativecontrols
- Timer
- Centrifuge

PRECAUTIONS

- Forprofessional*invitro*diagnosticuse only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch orcanister is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary stateof the animals does not completely guarantee the absence of transmissible pathogenic agents. It istherefore, recommended that these products be treated as potentially infectious, and handled byobserving usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for eachspecimenobtained.
- Readtheentireproceduracarefullypriortotesting.
- Do not eat, drink or smoke in the area where specimens and kits are handled. Handle all specimensasiftheycontaininfectiousagents.Observeestablishedprecautionsagainstmicrobiologicalhazardsthroughout the procedure and follow standard procedures for the proper disposal of specimens.Wear protective clothing such as laboratory coats, disposable gloves and eye protection whenspecimens are assayed.
- Humidityandtemperaturecanadverselyaffectresults.
- Usedtestingmaterialsshouldbediscardedinaccordancewithlocalregulations.

STORAGEANDSTABILITY

- Thekitshouldbestoredat2-30°Cuntiltheexpirydateprintedonthesealedpouch.
- Thetestmustremaininthesealedpouchorclosedcanisteruntil use.
- **Donotfreeze.**
- Kits should be kept out of direct sunlight.

SPECIMENCOLLECTIONAND STORAGE

- The COT Cotinine Test Device (Urine) is intended for use with human urine specimensonly.
- Urinecollectedatanytimeofthedaymaybeused.
- Urinespecimensmustbecollectedinclean,drycontainers.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and onlythe clear supernatantshould be used for testing.
- Performtestingimmediatelyafterspecimencollection.Donotleavespecimensatroomtemperaturefor prolonged periods. Urine specimens may be stored at 2-8°C for up to 2 days. For long termstorage, specimens should be kept below -20°C.
- Bringspecimenstoroomtemperaturepriortotesting.Frozenspecimensmustbecompletelythawedand mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations fortransportation of etiological agents.

PROCEDURE

Bringtests,specimensand/orcontrolstoroomtemperature(15-30°C)beforeuse.

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealedpouch 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 fulldropsofurine**(approx.100µL)tothespecimenwell(S)ofthetestdevice,andthenstartthetimer.Avoid trapping air bubbles in the specimen well (S).
3. Waitforthecoloredline(s)toappear.**Readresultsat5minutes**.Donotinterprettheresultafter10minutes.

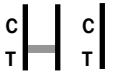
INTERPRETATIONOFRESULTS



POSITIVE: Only one colored band appears, in the control region (C). Noapparent colored band appears in the test region (T).



NEGATIVE: Two colored bands appear on the membrane. One band appears inthe control region (C) and another band appears in the test region (T).



INVALID: Control band fails to appear. Results from any test which has notproduced a control band at the specified read time must be discarded. Please reviewthe procedure and repeat with a new test. If the problem persists, discontinue usingthe kit immediately and contact your local distributor.

NOTE:

1. The intensityof color in the test region (T)mayvarydependingon theconcentrationof analytes presentinthespecimen.Therefore,anyshadeofcolorinthetestregionshouldbeconsiderednegative.Notethatthis a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likelyreasons for control band failure.

QUALITYCONTROL

- Internalproceduralcontrolsareincludedinthetest.Acoloredbandappearing inthecontrolregion (C)isconsideredaninternalpositiveproceduralcontrol,confirming sufficientspecimenvolumeand correct procedural technique.
- Externalcontrolsarenotsuppliedwiththiskit.Itisrecommendedthatpositiveandnegativecontrols be tested as a good laboratory practice to confirm the test procedure and to verify proper

test performance.

LIMITATIONSOFTHETEST

1. The COT Cotinine Test Device (Urine) is for professional *in vitro* diagnostic use, andshould be only used for the qualitative detection of Cotinine.
2. This assay provides a preliminary analytical test result only. A more specific alternative chemicalmethod must be used in order to obtain a confirmed analytical result. Gas chromatography/massspectrometry (GC/MS) has been established as the preferred confirmatory method by the NationalInstituteonDrugAbuse(NIDA).Clinicalconsiderationandprofessionaljudgmentshouldbe
- Care should be taken to protect the components of the kit from contamination. Do not use if there isevidence of microbial contamination or precipitation. Biological contamination of dispensingequipment, containers or reagents can lead to false results.

CotinineTestDevice(Urine)identifiedpositiveresultsat5minutes.

Cotininerelatedcompounds	Concentration(ng/ml)
Cotinine	200
Buprenorphine	> 50.000

Thefollowing compounds yieldednegative results upto a concentrationof 100 µg/mL:

Acetaminophen	Dihydrocodeine	Nalorphine
Acetophenetidine	(+)-cis-Diltiazem	Naloxone
Acetylcodeine	Dimenhydrinate	(+)-NaproxenAcetylsalicylicacid
4-Dimethylaminoantipyrine	NifedipineAlprazolam	Diphenhydramine
Nimesulide	Amikacin	DL-Tryptophan
Nitrazepam	Aminopyrine	DL-Tyrosine
Olanzapine	Amitriptyline	Dopamine
Opipramol	Amoxicilline	Doxepin
Oxalicacid	Amphetamine	Doxylamine
Oxazepam	Ampicilline	d-Propoxyphene
Oxycodone	Apomorphine	EcgonineHCl
Oxymetazoline	Ascorbicacid	Ecgoninemethylester
PennicillineG	Aspartame	Ephedrine
Perphenazine	Atropine	(+/-)Epinephrine
Pheniramine	Baclofen	Erythromycine
Phenothiazine	Benzocaine	Estron3sulfate
Phentermine	Bilirubin	Ethylmorphine
(+/-)Phenylpropanolamine	Bromazepam	Etodolac
beta-phenylethylamine	Caffeine	Fenfluramine
Prednisolone	Cannabidiol	Fentanyl
Prednisone	Cannabinol	Flupentixol
Phencyclidine	Carbamazepine	Fluoxetine
Procaine	Chloramphenicol	Furosemide
Promazine	Chlordiazepoxide	Gastrozepin
Promethazine	Chloroquine	Gentamicin
Prothipendyl	Chlorpheniramine	Gentisic acid
Protriptyline	Chlorprothixene	GuaiacolGlycerylEther
Quetiapine	Cholesterol	Hemoglobin
Quinidine	Chorptothixene	Hydralazine
Ranitidine	Cimetidine	Hydrochlorothiazide
Rifampicine	Ciprofloxacin	Hydrocodone
Risperidone	Citalopram	Hydrocortisone
Salbutamol	Clindamycin	Ibuprofen
Salicylicacid	Clobazam	Imipramine
Secobarbital	Clomipramine	(-)Isoproterenol
Sertraline	Clonazepam	Ketamine
Spironolactone	Clonidine	Ketoprofen
Sulfamethoxazole	Clorazepate	L-Thyroxine
Sulindac	Clozapine	Lincomycin
Temazepam	Cocain	Lidocaine
Thebaine	Codein	Loperamide
Theophylline	Creatinine	L-Phenylephrine
Thiamine	Cyclobenzaprine	Maprotiline
Thioridazine	Delorazepam	Meperidine
TobramycinDesipramineHCl	Mephentermine hemisulfate salt	TriamtereneDexamethasone
Methadone	Trimethoprim	Dextromethorphan
Methamphetamine	TrimipramineDiacetylmorphine	3,4-Methylenedioxyamphetamine
Tyramine	Diazepam	3,4-Methylenedioxy-methamphetamine
Vancomycin	Diclofenac	N-Methylephedrine
Venlafaxine	Dicumarol	Metoclopramide
Verapamil	Diflunisal	Metoprolol
Zolpidem	DL-PropanololDigoxin	Metronidazole
MOR-3-Beta-DGlucuronide		

LITERATUREREFERENCES

appliedtoanytestresult,particularlywhenpreliminarypositiveresultsareindicated.

3. There is a possibilitythat technical or procedural errors as well as other substances and factors mayinterfere with the test and cause false results.
4. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous resultsregardless of the analytical method used. Therefore, please preclude the possibility of urineadulteration prior to testing.
5. A positive result indicates the presence of a Cotinine only, and does not indicate or measureintoxication.
6. A negative result does not at any time rule out the presence of Cotinine in urine, as they may bepresent below the minimum detection level of the test.
7. Thistestdoesnotdistinguishbetween Cotinineandcertain medications.

PERFORMANCECHARACTERISTICS

A. Accuracy

The accuracy of the COT Cotinine Test Device (Urine) was compared and checked againstcommerciallyavailable tests with a threshold value at the same cut-off levels. Urine samples taken fromvolunteers claiming to be non-users were examined under both tests. The results were >99.9% inagreement.

B. Reproducibility

The reproducibility of the COT Cotinine Test Device (Urine) was verified by blind testsperformed at four different locations. Samples with Cotinine concentrations at 50% of the cut-off wereall determined to be negative, while samples with Cotinine concentrations at 200% of the cut-off wereall determined to be positive.

C. Precision

Test precision was determined by blind tests with control solutions. Controls with

Cotinineconcentrations at 50% of the cut-off yielded negative results, and controls with Cotinine concentrationsat 150% of the cut-off yielded positive results.

D. Specificity

Thefollowingtableslisttheconcentrationsofcompounds(ng/mL)abovewhichtheCOTOneStep

1. BaseltRC.DispositionofToxicDrugsandChemicalsinMan.2nded.Davis:BiomedicalPublications;1982.
2. HawksRL,ChiangCN,eds.UrineTestingforDrugsofAbuse.Rockville:DepartmentofHealthand Human Services, National Institute on Drug Abuse; 1986.
3. Substance Abuse and Mental Health Services Administration. Mandatory Guidelines for FederalWorkplace Drug Testing Programs. 53 Federal Register; 1988.
4. McBay AJ. Drug-analysis technology--pitfalls and problems of drug testing. Clin Chem. 1987Oct; 33 (11 Suppl): 33B-40B.
5. Gilman AG, Goodman LS, Gilman A, eds. Goodman and Gilman's The Pharmacological Basisof Therapeutics. 6th ed. New York: Macmillan; 1980.

IndexofSymbols

	Consultinstructionsforuse		Testsperkit		AuthorizedRepresentative
	For*in vitro* diagnosticuseonly		Useby		Donot reuse
	Storebetween2-30°C		Lot Number		Cataloguenumber

Mfd by: BIOBOON BIOTECH INC, 90 East Halsey Road Ste. 333 #434 Parsippany, New Jersey 07054, U.S.A.
Email: info@bioboonbiotech.com