Non Cross-Reacting Compounds

4-Acetaminophenol	Acetone
Acetophenetidin	Acetylsalicyclic Acid
Albumin	Aminopyrine
Amitryptyline	Amobarbital
Amoxapine	Amoxicillin
Ampicillin	Apomorphine
Aspartame	Atropine
l-Amphetamine	l-Ascorbic acid
Benzilic acid	Benzoic acid
Benzoylecgonine	Benzphetamine
B-Estradiol	Bilirubin
Brompheniramine	Buspirone
(+)-Chlorpheniramine	(±)Chlorpheniramine
Caffeine	Cannabidiol
Cannabinol	Chloral Hydrate
Chloramphenicol	Chlordiazepoxide
Chloroquine	Chlorpromazine
Chlorprothixene	Cholestrol
Cimetidine	Clomopramme
Clonidine	Cocaine
Codeine	Cortisone
Creatinine	Cyclobarbital
Cyclobenzaprine	(-)Deoxyephedrine
4-Dimethylaminoantipyrine	5,5Diphenylhydantoin
Deoxycorticosterone	Dextromethorphan
Diazepam	Diclofenax
Digoxin	Diphenhydramine
Disopyramide	Doxylamine
R(-)Deprenyl	(-)Ψ- Ephedrine
(±)Epinephrine	(tr,2s)-(-)Ephedrine
Ecgonine	Ecgonine Methylester
EDDP	Efavirenz (Sustiva)
EMDP	Ephedrine
Erythromycin	Estrone 3-sulfate
Ethanol	Ethyl-p-aminobenzoate
Etodolac	l-Ephedrine
l-Epinephrine	Famprofazone
Fenfluramine	Fenoprofen
Fentanyl	Fluoxetine
Furosemide	d(-)Glucose
Gentisic acid	Guaiacol Glyceryl Ether
3-Hydroxytyramine	Hemoglobin
Hydralazine	Hydrochlorothiazide
Hydrocodone	Hydrocortisone
Hydromorphone	Hydroxyzine
o-Hydroxyhippuric acid	p-Hydrixymethamphetamine
p-Hydroxyamphetamine	p-Hydroxynorephedrine
(-)Isoproterenol	Ibuprofen
Imipramine	Iproniazid
Isozsuprine	Kanamycin
Ketamine	Ketoprofen
Labetalol	Levorphanol
Lidocaine	Lindane
Lithum Carbonate	Loperamine
d-Methamphetamine	l-Methamphetmine
Maprotiline	MDA*
MDMA**	Meperidine
Mephentermine	Meprobatnate
Methadone	Methaqualone
Methoxyphenamine	Methyprylon
Metoprolol	Morphine 3-β-d-glucuronide
Morphine Sulfate	Mythlphenidate
a-Naphthaleneactetic acid	d-l Norephedrine
d-Norpropoxyphene	Nalidixic acide
Nalorphine	Naloxone
Naltrexone	Nimesulide
Norcodeine	Norethindone
Normorphine	Noscapine
D,l-Octopamine	Orphenadrine
Oxalic acid	Oxazepam
Oxilinic acid	Oxycodone
Oxymetalozine	Oxymorphone
(±)Phenylpropanolamine	B-Phenylethylamine
D,l-Propanolol	d-Propoxyphene
d-Pseudoephedrine	l-Phenylephrine
Papaverine	Pemoline
Penicillin-G	Pentazocine
Pentobarbital	Perphenazine
Phencyclidine	Phenelzine
Pheniramine	Phenobarbital
Phenothiazine	Phentermine
Prednisolone	Prednisone
Procaine	Promothazine
Protnazine	Trans-2-phenylcyclopropylamine
Quinacrine	Quinidine
Quinine	Ramtidine
Riboflavin	Salicyclic acid
Secobarbital	Serotonin
Sodium Chloride	Sulfamethazine
Sulindac	Temazepam
Tetracycline	Tetrahydrocortisone
3-acetate Tetrahydrozoline	Thebane
Theophylline	Thamine
Thiodrizadine	l-Thyroxine
Trifluoperazine	Trimethobenzamide
Thrumethoprim	Trimipramine
d.l-Tryptophan	Tyramine
D,l-Tyrosine	Uric Acir
Verapmil	Zomepirax

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

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AccuTest™ NIC Test Cassette

FOR THE QUALITATIVE ASSESSMENT OF NICOTINE AND THE METABOLITES IN HUMAN URINE

For in vitro Diagnostic and Forensic Use

INTENDED USE

The AccuTest™ NIC test device is an immunochromatography based one step in vitro test. It is designed for qualitative determination of drug substances in human urine specimens. This assay may be used in the point of care setting. The cut-off concentration using our test is as follows;

Nicotine	200 µg/ml of cotinine

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. The Substance Abuse Mental Health Services Administration (SAMHSA) has established gas chromatography/ mass spectrometry (GC/MS) as 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 indicated.

Note: ***It is not for use in diagnosing disease or illness; it is for investigational use only.***

SUMMARY AND EXPLANATION

Smoking has been identified as a major risk factor for lung cancer, and cardiovascular disease.[1,2] Self-reporting of smoking status is not reliable.[3] The determination of cotinine, a major metabolite of nicotine, has become the preferred biomedical method of assessing the smoking status of individuals on account of its sensitivity and specificity.[4]

Cotinine is present in blood, urine and saliva of individuals who smoke or chew tobacco or who inhale tobacco smoke produced by others. As an objective indicator of nicotine intake or confirmation of nonsmoker status, cotinine offers several advantages over other biochemical measures: it is a specific indicator of nicotine intake, its concentrations are not influenced by confounding factors such as diet or environment, its average biological half-life in blood is 19 hours, and its concentration within a given individual varies by on 15 to 20% over the course of a day.[5] Cotinine assay is thus a superior objective measure of exposure to nicotine.

PRINCIPLE

The AccuTest™ NIC test device is based on the principle of specific immunochemical reaction between antibodies and antigen to analyze particular compound in human urine specimen. The assay relies on the competition for binding antibody. When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody dye conjugate. When the amount of drug is equal or more than the cut-off, it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

MATERIAL PROVIDED

1. An AccuTest™ NIC test device (cassette).
   The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-mouse IgG antibody.
   Test zone: contains drug bovine protein antigen conjugates

Control zone: contains Goat anti-mouse IgG antibody
Conjugate pad: contains mice monoclonal anti-drug antibody.
2. Dropper
3. Instruction for use.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Urine collection container.
2. Timer or clock.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic and professional use only.
2. Do not use the test device beyond the expiration date.
3. Urine specimens may be infectious; properly handle and dispose of all used reaction devices in a biohazard container.
4. Visually inspect the foil package to insure it is intact. If the package is not intact, the integrity of the device might be compromised.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

STORAGE AND STABILITY

The test device should be stored at 2°C to 28°C; **do not freeze** and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

SPECIMEN COLLECTION AND PREPARATION

Fresh urine does not require any special handling or pretreatment. Specimen should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2- 8 °C or frozen up to 7 days. Specimens should be brought to room temperature before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.

QUALITY CONTROL

Good Laboratory practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within establish range, assay results are invalid. Control materials that are not provided with this test kit are commercially available.

The AccuTest™ NIC urine screen test device provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PROCEDURE

1. Bring all materials and specimens to room temperature.
2. Remove the test strip from sealed foil pouch.
3. Place the sample pad end of the test strip (up to the bottom blue line mark) into the urine specimen.
4. Hold the strip in the urine for 10 seconds, remove from the urine.
5. At 5 minutes, read the test strip.

Do not interpret the result after 5 minutes. Waiting more than five minutes may cause the reading to be inaccurate. To avoid confusion, discard the test strip after interpreting the result.

INTERPRETATION OF RESULTS

Negative:

Two colored bands form on any strip of the card. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative result for that particular test(s). The negative result does not indicate the

absence of drug in the specimen; it only indicates the level of tested drug in the specimen is less than cut-off level**.**

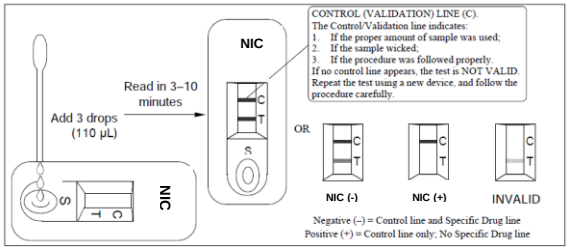
Positive:

One colored band appears in control line zone. No coloured band is found in test line zone. This is an indication the level of tested drug(s) in the specimen is above the cut- off level.

Invalid:

If there is no colored band in control line zone of any strip, the test result is invalid. Retest the sample with a new device.

Note: A borderline(+/-) in test line zone should be considered negative result.



LIMITATION OF PROCEDURE

The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication. There is a possibility that technical or processed urine error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer “SPECIFICITY” section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED RESULTS

The AccuTest™ NIC test device is a qualitative assay. It identifies the drug in human urine at its cut-off concentration or higher. The concentration of the drug cannot be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERFORMANCE CHARACTERISTICS

Accuracy:

A side-by-side comparison was conducted using the AccuTest™ NIC 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 tabled;-

METHOD		Other COT Device		TOTAL RESULTS
AccuTest™ NIC Test	Results	Positive	Negative	
	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: 0µg/mL, 100 µg/mL, 200 µg/mL, 250 µg/mL, 300 µg/mL and 400 µg/mL. The result demonstrates >99% accuracy at 100% above and 50% below the cut-off concentration. The data is summarized within the following table:-

Cotinine Concentration (µg/mL)	Percent of Cut-off	n	Visual Results	
			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 Sensitivity

The following table lists compounds that are positively detected in urine by the AccuTest™ NIC test device (urine) at 5 minutes

Compound	Concentration (µg/mL)
(-)-Cotinine	200
(-)-Nicotine	6,500

Precision

A study 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 200µg/mL cut-off level were used. The following results were tabled:-

Cotinine Concentration (µg/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

Effects of Urinary Specific Gravity

Fifteen urine specimens of normal, high and low specific gravity ranges were spiked with 100 µg/mL and 400 µg/mL. The AccuTest™ NIC test device (urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urine specific gravity do not affect the test results.

Effects of Urinary pH

The pH of an aliquot negative urines pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with Cotinine to 100 µg/mL and 400 µg/mL. The spiked, pH-adjusted urine was tested with the AccuTest™ NIC test device (urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test device.

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 AccuTest™ NIC test device at a concentration of 100µg/mL.