Accutest Saliva Cotinine Test
Package Insert

A rapid, screening test for the simultaneous, qualitative detection of Cotinine in human oral fluid.

For professional in vitro diagnostic use only,
For determination of nicotine (cotinine) use only.

INTENDED USE & SUMMARY

The Accutest Saliva Cotinine Test is a lateral flow chromatographic immunoassay for the qualitative detection of cotinine in oral fluids at a cut-off concentrations of 30 ng/mL.

Test	Calibrator	Cut-off (ng/mL)
Cotinine (COT)	Cotinine	30

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/mass spectrometry (GC/MS) and gas chromatography/tandem mass spectrometry (GC/MS/MS) are the preferred confirmatory methods. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY

The Accutest Saliva Cotinine Test is a rapid, oral fluid screening test that can be performed without the use of an instrument. The test utilizes antibodies to selectively detect elevated levels of specific drugs in human oral fluid.

Cotinine is the first-stage metabolite of nicotine, a toxic alkaloid that stimulates the autonomic ganglia and central nervous system 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. Aside from tobacco, nicotine is also commercially available as the active ingredient in smoking replacement therapies such as nicotine gum, transdermal patches and nasal sprays. Regardless of whether nicotine in a donor was derived from tobacco use or through a nicotine-replacement therapy, if the metabolite cotinine is present in sufficient concentration, the test result will be positive.

Although nicotine is excreted in saliva, the relatively short half-life of the drug makes it an unreliable marker for tobacco use. Cotinine, however, demonstrates a substantially longer half-life than nicotine, bears a high correlation with plasma cotinine levels and has been found to be the best marker for smoking status compared with saliva nicotine measurements, breath carbon monoxide testing and plasma thiocyanate testing1. The window of detection for cotinine in saliva at a cutoff level of 30 ng/mL is expected to be up to 1-4 days after nicotine use.

PRINCIPLE

The Accutest Saliva Cotinine Test is an immunoassay based on the principle of competitive binding. Drugs that may be present in the oral fluid specimen compete against their respective drug conjugates for binding sites on their specific antibody.

During testing, a portion of the oral fluid specimen migrates upward by capillary action. A drug, if present in the oral fluid specimen below its cut-off concentration, will not saturate the binding sites of its specific antibody. The antibody will then react with the drug-protein conjugate and a visible colored line will show up in the test line region of the specific drug strip. The presence of drug above the cut-off concentration in the oral fluid specimen will saturate all the binding sites of the antibody. Therefore, the colored line will not form in the test line region.

A drug-positive oral fluid specimen will not generate a colored line in the specific test line region of the strip because of drug competition, while a drug-negative oral fluid specimen will generate a line in the test line region because of the absence of drug competition.

To serve as a procedural control, a colored line will always appear at the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains membrane strips coated with drug-protein conjugates on the test line, polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with antibody specific to Cotinine.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date.
- The oral fluid test cube should remain in the sealed pouch until use.
- Saliva is not classified as biological hazard unless derived from a dental procedure.
- The used collector and cube 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

The oral fluid specimen should be collected using the collector provided with the kit. Follow the detailed Directions for Use below. No other collection devices should be used with this test. Oral fluid collected at any time of the day may be used. If specimen cannot be tested immediately, it is recommended that specimen be stored at 2-8°C or -20°C for up to 72 hours. Specimen may also be stored at room temperature for up to 48 hours. For ideal shipment conditions, transport specimen using ice packs (2-8°C).

MATERIALS

Materials Provided

- Test mini cubes
- Saliva collectors
- Security seal labels
- Package insert

Materials Required But Not Provided

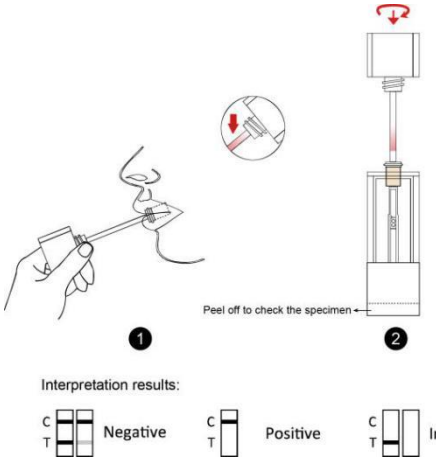
- Timer
- Gloves

DIRECTIONS FOR USE

Allow the test device, specimen, and/or controls to reach room temperature (15-30ºC) prior to testing. Instruct the donor to not place anything in the mouth including food, drink, gum, tobacco products for at least 10 minutes prior to collection.

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. Using the provided collection swab, remove the collector from the sealed pouch, have donor sweep inside of mouth (cheek, gum, tongue) several times, then hold swab in mouth until color on the saturation indicator strip appears in the indicator window dof collection swab. **Important:** Do not bite, suck, or chew on the sponge. **Note:** If after 7 minutes, color on the saturation indicator has not appeared in the indicator window, proceed with the test below. (See illustration 1)
3. Place the test device on a clean and flat surface. Remove the collection sponge from the mouth and screw the sponge first into the screening device **gently and slowly**, rotate until the collector cap sealed with the device tightly. **Keep upright when insert and press the sponge.** (See illustration 2)
4. **Test device upright on flat surface and keep upright while test is running.** Wait for the colored signal to appear in test results area. Read the results at 10 minutes.
5. **If positive results are observed, secure cap with security seal and send the device to a laboratory for confirmation. The laboratory can access the reservoir through the Sample Port.**

Note: 1, Once the collection sponge locks in place, the device is airtight, tamper evident, and ready to be disposed or sent to lab for confirmation (on presumptive positive result).
2, If no wicking issue occurred, please peel off the label at the bottom of the device as marked to check if there is enough specimen (obviously specimen residue) or the saliva is too thick or viscous to run.
3, In the case of no flowing even with enough saliva specimen, or the saliva is too thick to run, please move the device but don't tilt and keep upright back and forth on a flat and clean surface for several times until the saliva flows up (please peel off the specimen label to easily check and make sure the oral fluid can touch the strips to run). Do not tilt the device when the test is running before reading results.



INTERPRETATION OF RESULTS

(Please refer to the previous illustration)

NEGATIVE:* A colored line in the control line region (C) and a colored line in the test line region (T) indicate a negative result. This indicates that the drug concentration in the oral fluid specimen is below the designated cut-off level for that specific drug.

***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: A colored line in the control line region (C) but no line in the test line region (T) indicates a positive result. This indicates that the drug concentration in the oral fluid specimen exceeds the designated cut-off for that specific drug.

INVALID: Control line (C) 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 region (C) is considered an internal 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 a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The **Accutest Saliva Cotinine Test** 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) or gas chromatography/tandem mass spectrometry (GC/MS/MS) is the preferred confirmatory method.
2. A positive test result does not indicate the concentration of drug in the specimen or the route of administration.
3. A negative result may not necessarily indicate a drug-free specimen. Drug may be present in the specimen below the cut-off level of the test.

PERFORMANCE CHARACTERISTICS

Accuracy

105 clinical saliva samples were analyzed by LC-MS and by the Accutest Saliva Cotinine Test. Each test was performed by three operators. Samples were divided by concentration into five categories: drug-free, less than half the cutoff, near cutoff negative, near cutoff positive, and high positive. Results were as follows:

% Agreement with Commercial Kit

Specimen	COT
Positive	97.8%
Negative	>99%
Total	99.0%

% Agreement with LC/MS*

Specimen	COT
Positive	97.8%
Negative	>99%
Total	99.0%

*The volume of some of the specimens was insufficient for LC/MS testing

Analytical Sensitivity

A PBS pool was spiked with drugs to target concentrations of ± 50% cut-off and ± 25% cut-off and tested with the Oral Fluid Cotinine Test Mini Cube. The results are summarized below.

Drug conc. (Cut-off range)	n	COT	
		-	+
0% Cut-off	90	90	0
-50% Cut-off	90	90	0
-25% Cut-off	90	89	1
Cut-off	90	45	45
+25% Cut-off	90	5	85
+50% Cut-off	90	0	90

Analytical Specificity

The following table lists the concentration of compounds (ng/mL) above which the Oral Fluid Cotinine Test Mini Cube identified positive results at 10 minutes.

Drug	Concentration (ng/ml)
(-) Cotinine	30
S(-)-Nicotine	3,000

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds spiked into drug-free PBS stock. The following compounds demonstrated no false positive results on the Oral Fluid Drug Test Mini Cube when tested at concentrations up to 100 μg/mL.

Interference

To determine whether common candies, drinks and oral hygiene products interfere with the Oral Fluid Cotinine Test Mini Cube, five (5) tobacco-free volunteers drank or used the following items as usual or as directed by the instructions of the item. Ten (10) minutes following the exposure, each donor was tested using the Oral Fluid Cotinine Test Mini Cube. The results of each test were read at 10 minutes. All specimens produced expected negative results, leading to the conclusion that none of the items consumed affect the results of the Oral Fluid Cotinine Test Mini Cube.

Cola
Orange Flavored Drink
Green Tea
Coffee
Lollipop
Toothpaste
Mouthwash
Milk
Gum
Beer

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds spiked into drug-free PBS stock. The following compounds demonstrated no false positive results on the Oral Fluid Cotinine Test Cube when tested with concentrations up to 10 μg/mL.

Acetaminophen
Acetophenetidin
Aminopyrine
Amoxicillin
Ampicillin
l-Ascorbic acid
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzphetamine
Bilirubin
d,l-Brompheniramine
Caffeine
Cannabidol
Chloralhydrate
Chloramphenicol
Chlorothiazide
d,l-Chloropheniramine
Chlorpromazine
Chloroquine
Cholesterol
Clonidine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diclofenac
Diflunisal
Digoxin
Diphenhydramine
l-Ψ-Ephedrine
β-Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
l-(-)-Epinephrine
Erythromycin
Fenoprofen
Furosemide
Gentisic acid
Hemoglobin
Hydralazine
Hydrochlorothiazide
Hydrocortisone
o-Hydroxyhippuric acid
p-Hydroxytyramine
Ibuprofen
Iproniazid
d,l-Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Loperamide
Meperidine
Meprobamate
Methylphenidate
Nalidixic acid
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norethindrone
d-Norpropoxyphene
Noscapine
d,l-Octopamine
Oxalic acid
Oxolinic acid
Oxymetazoline
Papaverine
Penicillin-G
Pentazocine
Perphenazine
Phenelzine
Trans-2-phenylcyclopropylamine
Phenylpropanolamine
Prednisolone
Prednisone
d,l-Propranolol
d-Propoxyphene
d-Pseudoephedrine
Quinacrine
Quinine
Quindine
Ranitidine
Salicylic acid
Serotonin
Sulfamethazine
Sulindac
Tetracycline
Tetrahydrocortisone 3-Acetate
Thiamine
Thioridazine
d,l-Tyrosine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
d,l-Tryptophan
Tyramine
Uric acid
Verapamil
Zomepirac

BIBLIOGRAPHY

1. Cone, EJ, “Saliva Testing for Drugs of Abuse,” Ann NY Acad Sci, 1993; 694: pp120

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