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*i*Screen® OFD Cotinine Test Device

Package Insert for the COT Test for Oral Fluids

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

For professional in vitro diagnostic use only.

INTENDED USE

The Alere *i*Screen® OFD Cotinine Test Device is a lateral flow chromatographic immunoassay for the qualitative detection of cotinine in oral fluids at a cut-off concentrations of 30 ng/mL.

This assay provides only a preliminary analytical test result. 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), gas chromatography/tandem mass spectrometry (GC/MS/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) are the preferred confirmatory methods.

SUMMARY

The Alere *i*Screen® OFD Cotinine Test Device 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 (COT)

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 testing[1]. 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.

ASSAY PRINCIPLE

The Alere *i*Screen® OFD Cotinine Test Device 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

- The device is **for professional *in vitro* diagnostic use only**. Do not use after the expiration date.
- The oral fluid test device 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 device should be discarded according to federal, state and local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C. The test is stable through the expiration date printed on the sealed pouch. The test devices 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, following the detailed instructions under Directions for Use. No other collection devices should be used with this assay. Oral fluid collected at any time of the day may be used.

MATERIALS

Materials Provided

- Test devices
- Caps
- Sponge protectors
- Procedure cards
- Security seals
- Package insert

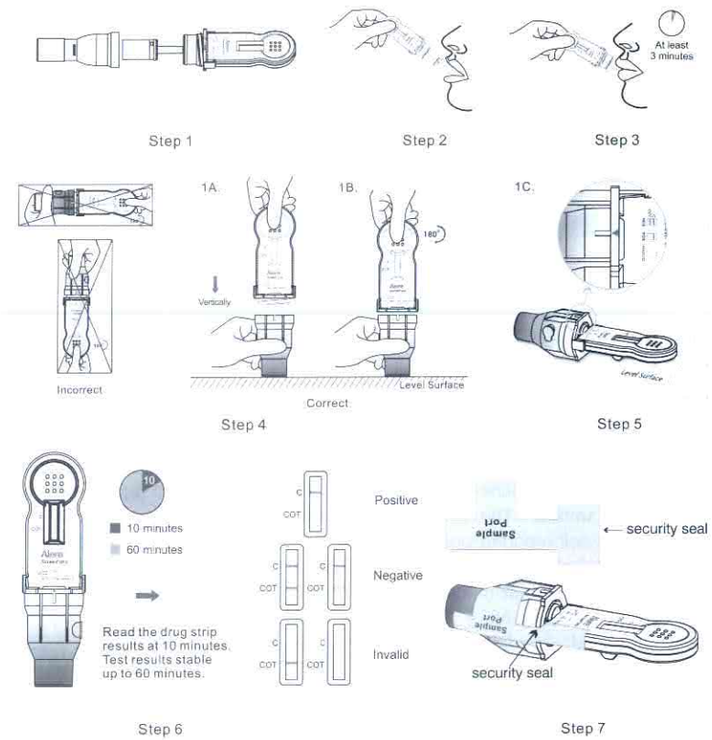
Materials Required but not Provided

- Timer

DIRECTIONS FOR USE

Allow the Alere *i*Screen® OFD Cotinine Test Device to come to room temperature [15-30°C (59-86°F)] prior to testing. Instruct the donor not to place anything in the mouth including food, drink, gum, or tobacco products for at least 10 minutes prior to collection.

1. Bring the pouch to room temperature before opening it. Remove the test and Cap from the sealed pouch and use the test as soon as possible.
2. Remove the Sample Collector Protector from the collection Sponge. Instruct the donor to insert the Sponge end of the collector into the mouth and actively swab the inside of the mouth and the top of the tongue. As soon as the Sponge softens slightly, the donor should gently press the Sponge between the tongue and teeth to ensure **complete saturation**.
3. The Sponge is saturated when no hard spots can be felt. Collect for a total of at least three (3) minutes before removing the Sponge. Remove the collector from the mouth.
4. Align the **Red Arrow** on the device with one of the **White Marks** on the Cap. Insert the collector **vertically** into the Cap and **press down firmly**. Twist the Cap clockwise 180° until the **Red Arrow** lines up with the other **White Mark**.
5. Place the test device horizontally on a clean and level surface with facing up.
6. **Read results at 10 minutes**. Do not read results after 1 hour.
7. If positive results are observed and confirmation is requested, secure Cap with the security seal and send the device to a laboratory. The laboratory can access the reservoir through the Sample Port.
8. For detailed operating instructions, please refer to the Procedure Card.



INTERPRETATION OF RESULTS

(Please refer to the previous illustration)

NEGATIVE:* All test lines appear. One colored line should be in the control region (C), and other apparent colored line should be adjacent in the test region (Drug/T). This negative result indicates that the drug concentration is below the detectable level or drug free.

***NOTE**: The shade of color in the test region (Drug/T) will vary, but it should be considered negative whenever there is even a faint colored line.

POSITIVE: One colored line appears in the control region (C). Any test line not appears in the test region (Drug/T). This positive result indicates that the drug concentration is above the detectable 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 using a new test panel. If the problem persists, discontinue using the lot immediately and contact the manufacturer.

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.

LIMITATIONS

1. The Alere *i*Screen® OFD Cotinine Test Device provides only a preliminary analytical test result. 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), gas chromatography/tandem mass spectrometry (GC/MS/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) are the preferred confirmatory methods.
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 cutoff level of the assay.

PERFORMANCE CHARACTERISTICS

Accuracy

The results outlined in the tables below represent unique specimens collected from thirty-nine (39) self-reported nicotine-free donors not exposed to secondhand smoke, six (6) self-reported nicotine-free donors who are exposed to secondhand smoke and forty-two (42) self-reported cigarette smokers. The specimens were testing using the Alere *i*Screen® OFD Cotinine Test Device and the Orasure Cotinine micro-plate EIA (each donor provided a specimen using the direct-collect Alere *i*Screen® OFD Cotinine Test Device and the Orasure Intercept collector provided in conjunction with the micro-plate EIA kit). Both assays showed 100% correlation of results for donors who were self-reported as nicotine-free including the six (6) donors who reported exposure to secondhand smoke. The two assays differed in the test results for two of the specimens from self-reported positive donors; the Alere *i*Screen® OFD Cotinine Test Device showed negative results while the Orasure micro-plate showed preliminary positive results. The specimens were determined by LC/MS to be below the cutoff level of the test, so the Alere *i*Screen® OFD Cotinine Test Device negative results correctly correlated with LC/MS. Both assays showed positive results for a specimen that was determined by LC/MS to contain 17 ng/mL of cotinine, so both assays were incorrect in that finding.

% Agreement with Commercial Kit

Specimen	COT
Positive	97.6%
Negative	>99%
Total	98.9%

% Agreement with LC/MS*

Specimen	COT
Positive	>99%
Negative	97.9%
Total	98.6%

*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 Alere *i*Screen® OFD Cotinine Test Device. 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 Alere *i*Screen® OFD Cotinine Test Device identified positive results at a read time of 10 minutes.

COTININE (COT)	
(-) Cotinine	30
S(-)-Nicotine	5,000

Interference

To determine whether common candies, drinks and oral hygiene products interfere with the Alere *i*Screen® OFD Cotinine Test Device, 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 Alere *i*Screen® OFD Cotinine Test Device. 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 Alere *i*Screen® OFD Cotinine Test Device.

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 Alere *i*Screen® OFD Cotinine Test Device when tested with concentrations up to 10 µg/mL.

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

BIBLIOGRAPHY

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

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