

Alere™ *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 healthcare professionals including professionals at point of care sites.

For 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. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. 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. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

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.

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 1-2 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

- For healthcare professionals including professionals at point of care sites.
- The device is **for 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

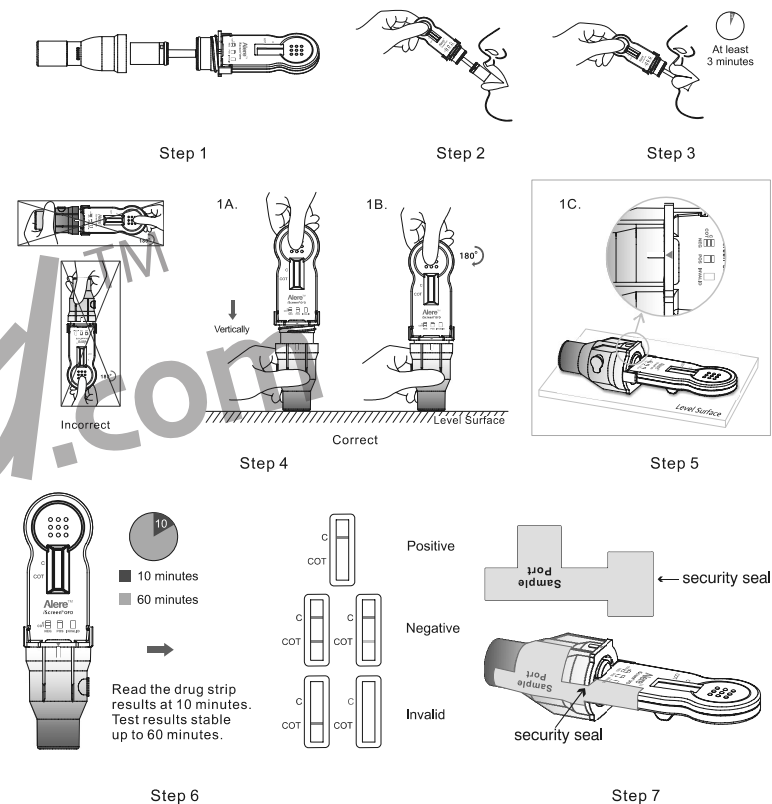
- Timer

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, secure Cap with security seal and send the device to a laboratory for confirmation. 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

- The **Alere iScreen® OFD Cotinine Test Device** 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), gas chromatography/tandem mass spectrometry (GC/MS/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) are preferred confirmatory methods.
- A positive test result does not indicate the concentration of drug in the specimen or the route of administration.
- 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

% 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%

Analytical Sensitivity

A PBS pool was spiked with drugs to target concentrations of $\pm 50\%$ cut-off and $\pm 25\%$ cut-off and tested with the **Alere iScreen® 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 iScreen® OFD Cotinine Test Device** identified positive results at a read time of 10 minutes.

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

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 **Alere iScreen® OFD Cotinine Test Device** when tested with concentrations up to 10 $\mu\text{g/mL}$.

Acetaminophen	Acetophenetidin
Aminopyrine	Amoxicillin
Ampicillin	l-Ascorbic acid
Apomorphine	Aspartame
Atropine	Benzilic acid
Benzoic acid	Benzphetamine
Bilirubin	d,l-Brompheniramine
Caffeine	Cannabidiol
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

Quinine
Salicylic acid
Sulfamethazine
Tetracycline
Thiamine
d,l-Tyrosine
Triamterene
Trimethoprim
Tyramine
Verapamil

Ranitidine
Serotonin
Sulindac
Tetrahydrocortisone 3-Acetate
Thioridazine
Tolbutamide
Trifluoperazine
d,l-Tryptophan
Uric acid
Zomepirac

BIBLIOGRAPHY

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