

INTENDED USE

The COT Rapid 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 the autonomic ganglia and central nervous system when 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. In addition to tobacco, nicotine is also commercially available as the active ingredient in smoking replacement therapies such as nicotine gum, transdermal patches and nasal sprays. In a 24-hour urine, approximately 5% of a nicotine dose is excreted as unchanged drug with 10% as cotinine and 35% as hydroxycotinine; the concentrations of other metabolites are believed to account for less than 5%. While cotinine is thought to be an inactive metabolite, it's elimination profile is more stable than that of nicotine which is largely urine pH dependent. As a result, cotinine is considered a good biological marker for determining nicotine use. The plasma half-life of cotinine is approximately 60 minutes following inhalation or parental administration. Nicotine and cotinine are rapidly eliminated by the kidney; the window of detection for cotinine in urine at a cutoff level of 200 ng/mL is expected to be up to 2-3 days after nicotine use.

PRINCIPLE

The COT Rapid Test Device (Urine) detects Cotinine through visual interpretation of color development on the device. Drug conjugates are immobilized on the test region of the membrane. During testing, the specimen reacts with antibodies conjugated to colored particles and precoated on the sample pad. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there are insufficient drug molecules in the specimen, the antibody-colored particle conjugate 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 drug molecules are present in the urine above the cut-off concentration of the test, they compete with the immobilized drug conjugate on the test region for limited antibody binding sites. This will prevent attachment of the antibody-colored particle conjugate to the test region. Therefore, the absence of a colored band at the test region indicates a positive result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

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

The control zone of each test contains goat anti-rabbit IgG antibody. The test zone of each test contains drug-bovine protein antigen conjugate, and the conjugate pad of each test contains monoclonal anti-drug antibody and rabbit antibody-colored particle complex.

MATERIALS
Materials Provided

- Individually packed test devices
- Package insert
- Disposable pipettes

Materials Required but Not provided

- Centrifuge
- Timer
- Positive and negative controls

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye

protection when specimens are assayed.

- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

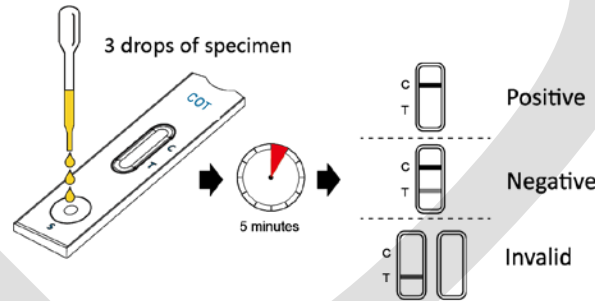
SPECIMEN COLLECTION AND STORAGE

- The COT Rapid Test Device (Urine) is intended for use with human urine specimens only.
- Urine collected at any time of the day may be used.
- Urine specimens must be collected in clean, dry containers.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Urine specimens may be stored at 2-8°C for up to 2 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and 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 for transportation of etiological agents.

PROCEDURE

Bring tests, specimens, and/or controls to room temperature (15-30°C) before use.

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the test with patient or control identification. For best results, the assay should be performed within one hour.
2. Using the provided disposable pipette, transfer 3 drops of specimen (approximately 120 µL) to the specimen well (S) of the device and start the timer.
Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.
As the test begins to work, color will migrate across the membrane.
3. Wait for the colored band(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes


INTERPRETATION OF RESULTS


POSITIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).



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



INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered negative. Note that this is 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 likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. 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 OF THE TEST

1. The COT Rapid Test Device (Urine) is for professional *in vitro* diagnostic use, and should be only used for the qualitative detection of Cotinine.
2. This assay provides a preliminary analytical test result only. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the National Institute on Drug Abuse (NIDA). Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.
3. There is a possibility that technical or procedural errors as well as other substances and factors may interfere with the test and cause false results.
4. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. Therefore, please preclude the possibility of urine adulteration prior to testing.
5. A positive result indicates the presence of a Cotinine only, and does not indicate or measure intoxication.
6. A negative result does not at any time rule out the presence of Cotinine in urine, as they may be present below the minimum detection level of the test.
7. This test does not distinguish between Cotinine and certain medications.

PERFORMANCE CHARACTERISTICS
A. Accuracy

The accuracy of the COT Rapid Test Device (Urine) was compared and checked against commercially available tests with a threshold value at the same cut-off levels. Urine samples taken from volunteers claiming to be non-users were examined under both tests. The results were >99.9% in agreement.

B. Reproducibility

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

C. Precision

Test precision was determined by blind tests with control solutions. Controls with Cotinine concentrations at 50% of the cut-off yielded negative results, and controls with Cotinine concentrations at 150% of the cut-off yielded positive results.

D. Specificity

The following tables list the concentrations of compounds (ng/mL) above which the COT Rapid Test Device (Urine) identified positive results at 5 minutes.

Cotinine 200 related compounds	Concentration (ng/ml)
(-)-Cotinine	200
Buprenorphine	100,000

The following compounds were found not to cross-react when tested at concentrations at 100 µg/ml.

(-)-Ephedrine	Chlorpheniramine	Oxalic Acid
(+)-Naproxen	Creatine	Penicillin-G
(+/-)-Ephedrine	Dextromethorphan	Pheniramine
4-Dimethylaminoantirine	Dextrophan tartrate	Phenothiazine
Acetaminophen	Dopamine	Procaine
Acetone	Erythromycin	Protonix
Albumin	Ethanol	Pseudoephedrine
Amitriptyline	Furosemide	Quinidine
Ampicillin	Glucose	Ranitidine
Aspartame	Guaiacol Glyceryl Ether	Sertraline
Aspirin	Hemoglobin	Tyramine
Benzocaine	Imipramine	Trimепazine
Bilirubin	(+/-)-Isoproterenol	Venlafaxine
b-Phenylethyl-amine	Metadone	Ibuprofen
Caffeine	Vitamin C (Ascorbic Acid)	Lidocaine
Chloroquine		

LITERATURE REFERENCES

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GLOSSARY OF SYMBOLS

ρ	Catalog number	θ	Temperature limitation
ι	Consult instructions for use	Λ	Batch code
Ι	In vitro diagnostic medical device	ε	Use by
μ	Manufacturer	Τ	Contains sufficient for <n> tests
σ	Do not reuse	Α	Authorized representative in the European Community
γ	CE marking according to IVD Medical Devices Directive 98/79/EC		



μ

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