Test Definition: NICOU

Nicotine and Metabolites, Random, Urine

Overview

Useful For

Monitoring tobacco use

Monitoring patients on nicotine-replacement therapy for concurrent use of tobacco products

Method Name

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

NY State Available

Yes

Specimen

Specimen Type

Urine

Specimen Required

Supplies: Sarstedt Aliquot Tube, 5 mL (T914)
Container/Tube: Plastic urine container
Submission Container/Tube: Plastic, 5-mL tube
Specimen Volume: 3 mL
Collection Instructions:
1. Collect a random urine specimen.
2. No preservative.

Forms

If not ordering electronically, complete, print, and send a Therapeutics Test Request (T831) with the specimen.

Specimen Minimum Volume

2 mL

Reject Due To

Gross hemolysis	OK
Gross icterus	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Urine	Refrigerated (preferred)	28 days	
	Ambient	28 days	
	Frozen	365 days	

Clinical & Interpretive

Clinical Information

Tobacco use remains the leading cause of preventable disease, disability, and death in the United States. Nicotine, coadministered in tobacco products such as cigarettes, pipes, cigars, or chew, is an addictive substance that causes individuals to continue use of tobacco despite concerted efforts to quit. Nicotine stimulates dopamine release and increases dopamine concentration in the nucleus accumbens, a mechanism that is thought to be the basis for addiction to drugs of abuse.

Nicotine is rapidly metabolized in the liver to cotinine, exhibiting an elimination half-life of 2 hours. Cotinine exhibits an apparent elimination half-life of 15 hours. Patients using tobacco products excrete nicotine in urine in the concentration range of 1000 to 5000 ng/mL. Cotinine accumulates in urine in proportion to dose and hepatic metabolism (which is genetically determined); most tobacco users excrete cotinine in the range of 1000 to 8000 ng/mL. Urine concentrations of nicotine and metabolites in these ranges indicate the subject is using tobacco or is receiving high-dose nicotine patch therapy.

In addition to nicotine and metabolites, tobacco products contain other alkaloids that can serve as unique markers of tobacco use. Two such markers are anabasine and nornicotine. Anabasine is present in tobacco products but not in nicotine replacement therapies. Nornicotine is present as an alkaloid in tobacco products and as a metabolite of nicotine. The presence of anabasine (>10 ng/mL) or nornicotine (>30 ng/mL) in urine indicates current tobacco use, irrespective of whether the subject is on nicotine replacement therapy. The presence of nornicotine without anabasine is consistent with use of nicotine replacement products. Heavy tobacco users who abstain from tobacco for 2 weeks exhibit urine nicotine values below 30 ng/mL, cotinine values below 50 ng/mL, anabasine levels below 2 ng/mL, and nornicotine levels below 2 ng/mL.

Passive exposure to tobacco smoke can cause accumulation of nicotine metabolites in nontobacco users. Urine cotinine has been observed to accumulate up to 20 ng/mL from passive exposure. Neither anabasine nor nornicotine accumulates from passive exposure.

Tobacco users engaged in programs to abstain from tobacco require support in the form of counseling, pharmacotherapy, and continuous encouragement. Occasionally, counselors may elect to monitor abstinence by biochemical measurement of nicotine and metabolites in a random urine specimen to verify abstinence. If results of biologic testing indicate the patient is actively using a tobacco product during therapy, additional counseling or intervention may be appropriate.

Quantification of urine nicotine and metabolites while a patient is actively using a tobacco product is useful to define the concentrations that a patient achieves through self-administration of tobacco. Nicotine replacement dose can then be tailored to achieve the same concentrations early in treatment to assure adequate nicotine replacement so the patient may avoid the strong craving they may experience early in the withdrawal phase. This can be confirmed by

measurement of urine nicotine and metabolite concentrations at a steady state (2-3 days after replacement therapy is started). Once the patient is stabilized on the dose necessary to achieve complete replacement and responding well to therapy, the replacement dose can be slowly tapered to achieve complete withdrawal.

Reference Values

Non-tobacco user with no passive exposure:
Nicotine <5.0 ng/mL

Cotinine
<5.0 ng/mL

Anabasine
<2.0 ng/mL

Nornicotine
<2.0 ng/mL

Interpretation

Urine nicotine in the range of 1000 to 5000 ng/mL with cotinine in the range of 1000 to 8000 ng/mL indicates the subject is either actively using a tobacco product or on high-dose nicotine patch therapy.

The presence of anabasine and nornicotine indicates a subject on patch therapy who is actively using a tobacco product.

Typical findings are as follows:

While using a tobacco product:
-Peak nicotine concentration: 1000 to 5000 ng/mL
-Peak cotinine concentration: 1000 to 8000 ng/mL
-Anabasine concentration: 10 to 500 ng/mL
-Nornicotine concentration: 30 to 900 ng/mL

Tobacco user after 2 weeks complete abstinence:
-Nicotine concentration: <30 ng/mL
-Cotinine concentration: <50 ng/mL
-Anabasine concentration: <2.0 ng/mL
-Nornicotine concentration: <2.0 ng/mL

Nontobacco user with passive exposure:
-Nicotine concentration: <20 ng/mL
-Cotinine concentration: <20 ng/mL
-Anabasine concentration: <2.0 ng/mL
-Nornicotine concentration: <2.0 ng/mL

Nontobacco user with no passive exposure:
-Nicotine concentration: <5.0 ng/mL
-Cotinine concentration: <5.0 ng/mL

-Anabasine concentration: <2.0 ng/mL
-Nornicotine concentration: <2.0 ng/mL

Cautions
Knowledge of time elapsed between last dose and specimen collection is important for interpretation of test results.

Clinical Reference
1. Moyer TP, Charlson JR, Enger RJ, et al. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolic profiles. Clin Chem 2002;48(9):1460-1471
2. Dale LC, Hurt RD, Hays JT. Drug therapy to aid in smoking cessation. Tips on maximizing patients' chances for success. Postgrad Med. 1998;104(6):75-78, 83-84
3. Rudasingwa G, Kim Y, Lee C, Lee J, Kim S, Kim S. Comparison of nicotine dependence and biomarker levels among traditional cigarette, heat-not-burn cigarette, and liquid e-cigarette users: Results from the Think Study. Int J Environ Res Public Health. 2021;18(9):4777. doi:10.3390/ijerph18094777
4. Sharma P, Sane N, Anand SD, Marimutthu P, Benegal V. Assessment of cotinine in urine and saliva of smokers, passive smokers, and nonsmokers: Method validation using liquid chromatography and mass spectrometry. Indian J Psychiatry. 2019;61(3):270-276. doi:10.4103/psychiatry.IndianJPsychiatry_61_18
5. AACC: Nicotine and cotinine. Testing.com; Updated February 2, 2023, Accessed October 7, 2025. Available at www.testing.com/tests/nicotine-and-cotinine/

Performance

Method Description
Nicotine and metabolites are extracted from urine by solid-phase extraction techniques. The extract eluate is quantified by high-performance liquid chromatography tandem mass spectrometry.(Moyer TP, Charlson JR, Enger RJ, et al. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolic profiles. Clin Chem. 2002;48[9]:1460-1471; Oh J, Park MS, Chun MR, et al. A simple and high-throughput LC-MS/MS method for simultaneous measurement of nicotine, cotinine, 3-OH cotinine, nornicotine, and anabasine in urine and its application in the general Korean population. J Anal Toxicol. 2022:46[1]:25-36. doi:10.1093/jat/bkaa177)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
2 to 5 days

Specimen Retention Time
2 weeks

Test Definition: NICOU

Nicotine and Metabolites, Random, Urine

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Superior Drive

Fees & Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

G0480

80323 (if appropriate for select payers)

Clinical Toxicology CPT Code Client Guidance

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NICOU	Nicotine and Metabolites, U	43125-4

Result ID	Test Result Name	Result LOINC® Value
82510	Nicotine	3854-7
21321	Cotinine	10366-3
21324	Nornicotine	33917-6
21323	Anabasine	33915-0