

# Nicotine and Related Compounds in Urine and Serum/Plasma

## *TO ASSESS THE USE OF OR EXPOSURE TO TOBACCO PRODUCTS*

### Test Highlights

- Detects nicotine, cotinine, and trans-3'-hydroxycotinine in serum
- Detects the above three compounds, plus normicotine and anabasine, in urine
- Allows the general categorization of specimen donors as unexposed non-tobacco users, passively exposed, abstinent users (greater than 2 weeks), and active users

### Clinical Background

- The use of tobacco products, particularly smoking, has been described as the leading preventable cause of death and disability in the United States, and is a worldwide problem. At the end of the 20th century, tobacco use in the U.S. was responsible for 430,000 deaths per year, representing 20 percent of all deaths, including those from natural causes.
- Adverse health effects are cancers of the respiratory system – lung, larynx, oral cavity, and esophagus; ischemic heart disease and atherosclerotic peripheral vascular disease; other pulmonary effects such as chronic obstructive pulmonary disease (which includes chronic bronchitis and emphysema), asthma, respiratory infections, and an overall decrease in pulmonary function. Smoking during pregnancy increases frequency of intrauterine growth retardation and subsequent low birth weight. Second-hand smoke produces effects in non-smokers in the home and the workplace, which are primarily pulmonary problems.
- The American Cancer Society's goals for 2015 are a reduction in incidence of tobacco-induced cancer by 25 percent and in deaths from tobacco initiated cancers by 50 percent<sup>1</sup>. Several intervention techniques are enjoying some success: cigarette package labeling, advertisement restrictions in the electronic media, laws regarding smoking in public places, and smoking cessation program improvements, including the use of nicotine replacement therapy.

### Indications for Ordering

Monitoring for tobacco use is, or may be, helpful in the following situations:

- Compliance with requirements in smoking cessation programs
- Candidates for orthopedic surgery (particularly spinal fusion), pulmonary therapy, and organ transplant programs
- Women on high-level estrogen therapy (increased risk for stroke and heart attack)
- Applicants for private health and life insurance (some employers deny employment to active smokers in order to reduce company health insurance costs)
- Identification of tobacco-using patients on drug therapy for a variety of health problems (nicotine and compounds may interfere with drug therapy)

- Experimental nicotine therapy in cognitive degeneration disorders, e.g. Alzheimer, Parkinson, and attention deficit/hyperactivity disorder (ADHD). These applications are in the early stage of experimentation and use<sup>2</sup>.
- A forensic purpose is in child custody cases. A non-custodial parent may be required by the court to be tested for tobacco product use as a condition of having visitation rights.

### Interpretation

ARUP clients order twice as many urine nicotine assessments as compared to serum/plasma nicotine assessments.

- Advantages of urine: the concentrations of nicotine and related substances are substantially higher in urine than in serum/plasma, which results in a higher positive rate in urine. Testing urine allows for the identification of anabasine, another tobacco plant alkaloid in addition to nicotine, which is present in low concentrations, not detectable in serum/plasma. Anabasine is not expected to be present in pharmaceutical grade nicotine. A urine specimen that contains equal to or greater than 3 ng/mL anabasine is not consistent with nicotine replacement therapy but rather, indicates tobacco use has occurred (active smoking). As such, the presence of anabasine in the urine collected from a patient managed with nicotine replacement therapy is strong evidence that the patient is also using tobacco products and may therefore be out of compliance with the treatment program. This is an important advantage of urine over serum/plasma. It also facilitates the identification of normicotine, so that along with nicotine, cotinine, and trans-3'-hydroxycotinine, an estimate of the nicotine body burden can be made.
- Advantages of serum/plasma: If the donor is a dialysis patient, a valid urine specimen cannot be obtained; then serum/plasma remains the choice. This specimen is also best for supporting pharmacokinetic studies.
- How long after smoking will a person remain positive by this test? There are many variables which influence the time between peak levels of nicotine and related compounds, and when they are no longer detected (less than 2 ng/mL): polymorphisms of the enzyme CYP2A6; racial/ethnic differences (African- and Chinese-Americans achieve higher levels than Caucasians, and may remain positive longer); nicotine half-life is ca. 1-4 hours, while cotinine is 12-22 hours; however, the terminal half-life is much longer, due to nicotine being sequestered in lipid stores in the body<sup>2</sup>. In practical terms, a smoker who abstains for more than 2 weeks will appear the same as an unexposed non-user in serum/ plasma; in urine, an active

smoker will remain higher than a person passively exposed after 2 weeks, and may take a few more weeks to reach the unexposed non-user category.

- To determine which of the four categories a donor is in, by concentrations of the three analytes in serum/plasma and the five in urine, see accompanying table of reference intervals adapted from Moyer et al.<sup>3</sup>

### Limitations

A number of variables contribute to donor categorization from a urine test: the wider range of concentrations encountered in urine; whether a donor is a light smoker (ca. 2-10 cigarettes per day), medium smoker (ca. 10-20), or heavy smoker (ca. 20-40); variations in the cigarette product used (concentration of nicotine, physical characteristics - e.g., pH, and the presence of a filter); and variations in smoking patterns (depth of inhalation, non-inhalation, sampling on weekdays versus weekends). Nevertheless, approximate categorization from a urine specimen, e.g., between active and abstinent 2 weeks, or non-user and passive exposure, still has clinical utility, if the clinician recognizes the variables which contribute to the uncertainty.

### Methodology

Internal standards of nicotine and related compounds are added to an aliquot of the specimen. All of these compounds are removed

from the aliquot by solid phase extraction, with multiple wash steps and eluted into autosampler vials. The clarified eluate is injected into a liquid-chromatograph-tandem mass spectrometer, where analytes and internal standards are ionized and separated in quadrupole 1 as selected precursor ions. These ions are fragmented in the collision cell to produce product ions, which are separated in quadrupole 2 and delivered to the photon multiplier, where the signal is magnified to produce chromatograms. Two transitions are obtained for each analyte. This confers an increased degree of specificity on the analysis, minimizing potential interferences which could reduce the certainty of the qualitative analysis or create an error in its quantitation. Qualitative identification and quantitation are accomplished by comparison of the specimen components to a standard curve of each analyte addressed in the assay. The method is analytically sensitive and highly specific.

### References

- Tobacco Use: United States, 1900-1999. *ONCOLOGY* 1999;Vol 13, No 12.
- Tutka P, Mosiewicz J, and Wielosz M. Review: Pharmacokinetics and metabolism of nicotine. *Pharmacological Reports* 2005;57, 143-153.
- Moyer TP, Charleston JR, Enger RJ, Dale LC, Ebert JO, Schroeder DR, and Hurt RD. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolite profiles. *Clinical Chemistry* 2002;48:9, 1460-1471.

#### Reference Interval (Urine)

	Unexposed non-tobacco user	Passive exposure	Abstinent user for greater than 2 weeks	Active tobacco product user
Nicotine	Less than 2 ng/mL	Less than 20 ng/mL	Less than 30 ng/mL	1000-5000 ng/mL
Cotinine	Less than 5 ng/mL	Less than 20 ng/mL	Less than 50 ng/mL	1000-8000 ng/mL
3-OH-Cotinine	Less than 50 ng/mL	Less than 50 ng/mL	Less than 120 ng/mL	3000-25000 ng/mL
Nornicotine	Less than 2 ng/mL	Less than 2 ng/mL	Less than 2 ng/mL	30-900 ng/mL
Anabasine	Less than 2 ng/mL	Less than 2 ng/mL	Less than 2 ng/mL	3-500 ng/mL

Adapted from *Clinical Chemistry* 2002;48:9, 1460-71.

#### Reference Interval (Serum or Plasma)

	Unexposed non-tobacco user	Passive exposure	Abstinent user for more than 2 weeks	Active tobacco product use
Nicotine	Less than 2 ng/mL	Less than 2 ng/mL	Less than 2 ng/mL	30-50 ng/mL
Cotinine	Less than 2 ng/mL	Less than 8 ng/mL	Less than 2 ng/mL	200-800 ng/mL
3-OH-Cotinine	Less than 2 ng/mL	Less than 2 ng/mL	Less than 2 ng/mL	100-500 ng/mL

Adapted from *Clinical Chemistry* 2002;48:9, 1460-71.

### Test Information

0092356

Nicotine & Metabolites, Urine

0092361

Nicotine & Metabolites, Serum or Plasma

For specific collection, transport, and testing information, refer to the ARUP Web site at [www.aruplab.com](http://www.aruplab.com).