population (3). In the south of Spain, where the AF508 mutation has an incidence of 43.5% (4), lower than in the rest of Spain (53.2%) (3), the incidence of these 11 mutations may be higher.

Used in conjunction with the 31 mutations analyzed with the CF Genetic Analysis assay from Applied Biosystems, the mutational analysis of the 11 mutations presented here could enhance the detection rate in Spanish and Mediterranean populations to ~80%.

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References


Rapid and Simple Tandem Mass Spectrometry Method for Determination of Serum Cotinine Concentration, Mark D. Kellogg,1* Jasna Behaderovic,1 Oneil Bhalala,2 and Nader Rafii1 1Department of Laboratory Medicine, Children’s Hospital, Harvard Medical School, Boston MA; 2Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA; * address correspondence to this author at: Department of Laboratory Medicine, Children’s Hospital, Harvard Medical School, 300 Longwood Ave., Boston MA 02155; fax 617-730-0383, e-mail mark.kellogg@childrens.harvard.edu

Tobacco use, primarily through cigarette smoking, continues to be a primary cause of preventable death worldwide. It has been well documented that exposure to tobacco smoke, which is very common, causes chronic lung and heart disease. Data from the 1988–1991 National Health and Nutrition Examination Survey data found that 87.9% of nonsmokers had detectable concentrations of serum cotinine (1). Because of the health risks associated with tobacco exposure, analysis of biomarkers of tobacco exposure has increased. Cotinine is the preferred serum biomarker for tobacco exposure (2–5).

Nicotine, a natural product in tobacco, is rapidly absorbed through the lungs into the pulmonary venous circulation and then to the heart and other body organs. It has a biological half-life of only 1 h and is rapidly excreted in the urine. As such, its use to measure tobacco exposure is limited. Nicotine is metabolized primarily via oxidation of the α-carbon to cotinine and N-oxidation of the pyrrolidine ring (6, 7). Cotinine accounts for ~90% of nicotine metabolites in serum and has a half-life of 10–40 h (8, 9). This relatively longer half-life makes it suitable for assessing exposure to cigarette smoke.

Validated methods for cotinine analysis in passive smoke assessment generally require large sample volumes, which are unsuitable for pediatric populations. Available methods that use smaller sample volumes and/or less complex extractions do not provide adequate assay sensitivity, thus precluding their use in assessing passive cigarette smoke exposure (10). We describe the development of a sensitive single-step extraction and rapid method for serum cotinine based on ion spray tandem mass spectrometry (MS/MS).

Cotinine was purchased from Sigma Chemical Co., N1-cotinine-methyl-d3 from Cambridge Isotope Laboratory, and HPLC-grade methanol, optima-grade ammonium hydroxide, and methylene chloride from Fisher Scientific. Mass spectrometry was conducted with a PE Sciex API 3000 triple quadrupole MS with a Turbolon Spray interface and Analyst software (Applied Biosystems). The liquid chromatography (LC) system consisted of a LC-10AT VP liquid chromatograph with a SIL-10AD autoinjector and a SCL-10A VP system controller (Shimadzu Scientific Institute, Inc.).

Stock solutions of cotinine and internal standard were each prepared to obtain a concentration of 100 mg/L in methanol. These solutions were found to be stable for at least 6 months when stored at 4 °C. The internal standard was prepared by dilution (1:2000) of the stock solution with 10 mL/L ammonia in methanol (1:99 by volume) to make a solution of 50 μg/L. This solution was stored in a flammable cabinet freezer and was stable up to 30 days. The stock calibrator solution was diluted (1:200) in methanol to obtain a working solution of 500 μg/L. This solution was then serially diluted to obtain the final calibrator concentrations of 200, 50, 20, 5, and 2 μg/L. Controls were prepared by adding stock solutions containing 500, 200, and 50 mg/L cotinine to pooled sera collected from smokers and nonsmokers at a volume ratio of 1:19. These solutions were then further diluted (1:100) with pooled serum, and the final concentrations of 268, 124, and 33 μg/L were determined by standard addition.

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Calibrator solutions and control aliquots were stored at 
−70 °C and were found to be stable up to 6 months. 
Monthly preparations of new solutions, which were run 
in parallel with existing solutions, were used to assess 
stability. Deviations >5% were considered significant. 
The mobile phase consisted of 100% methanol. Cotinine-
free serum for use in calibration was created by pooling 
sera from individuals with no known passive smoke 
exposure for at least 5 days and found to have serum 
cotinine <2 µg/L as measured by an independent labo-
ratory. New serum pools were tested against existing 
calibrators to verify that cotinine concentrations were less 
than the method detection limit of 0.6 µg/L.

A working diluent/internal standard solution was pre-
pared by combining 800 µL of internal standard, 5.2 mL of 
methanol, 16 µL of ammonium hydroxide, and 6 mL of 
distilled water. We mixed 50 µL of sample, control, or 
calibrator with 200 µL of the working diluent/internal 
standard and 400 µL of methylene chloride. We then 
added an additional 50 µL of methanol to control and 
patient samples, and 50 µL of cotinine-free serum to the 
calibrator. All samples were vortex-mixed for 10 s and 
centrifuged at 16 000g for 1 min. We transferred 300 µL of 
the lower methylene chloride layer to a clean glass vial. A 
30-µL injection was made into the tandem mass spectrom-
eter, equipped turbo spray ion interface, at a flow rate of 
0.2 mL/min; the total analysis time was 3 min. The 
tandem mass spectrometer was used in the positive-ion 
mode. Nitrogen was used as the drying gas at a flow rate 
of 8 L/min and for collision-activated dissociation. The 
ion source temperature was 450 °C, the declustering po-
tential was 41 V, and the collision energy was 33 eV. 
Cotinine was detected in the multiple-reaction-monitor-
ing mode at a m/z 177/80 and 177/98 transitions. Analyst 
software, Ver. 1.2 (Applied Biosystems/MDS SCIEX), was 
used for system control, data acquisition, and data pro-
cessing.

The assay was linear to a cotinine concentration of 500 
µg/L. The lower limit of detection was 0.54 µg/L, based 
on +2 SD from the mean of 20 replicates of drug-free sera 
(0.008 ± 0.271 µg/L) and 20 replicates of the 2 µg/L 
calibrator (2.02 ± 0.19 µg/L). Data were evaluated with 
the EP Evaluator Online statistics program (David G. 
Rhoads Associates, Inc.). Functional sensitivity (interas-
say CV <20%) of the method is 1.4 µg/L. Between-run 
imprecision (CV) at cotinine concentrations of 2, 5, 20, 33, 
50, 124, and 268 µg/L was 11%, 9.5%, 6.1%, 7.2%, 4.0%, 
5.6%, and 2.6% (n = 20), respectively. Recovery in serum 
samples to which cotinine had been added at concentra-
tions of 2–300 µg/L ranged from 92% to 103%. To further 
assess the accuracy of the method, we assayed 40 samples 
previously analyzed by LC-MS/MS at Mayo Clinic Lab-
oratories (Rochester, MN and Wilmington, MA) (11). The 
results are summarized in Fig. 1.

To assess interference, we assayed combinations of 51 
different drugs and ascorbic acid. We mixed 20 µL of the 
drug cocktail with 50 µL of serum with a known cotinine 
concentration of 20 µg/L. Final concentrations of the 
tested substances were ~1 mg/L. Samples were then
extracted and tested as described above. None of the substances produced an interference >5% compared with the serum diluted with drug cocktail diluent. The list of drugs tested appears in Supplement 1, which accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue11/.

Ion suppression was assessed as recommended by Annesley (12). Briefly, the response of calibrators injected into the mobile phase was compared with the same amount of cotinine added to unextracted and extracted samples. To assure that slight differences in matrix were not causing suppression, we used 20 different cotinine-free serum samples. We observed a small decrease in signal, but because the internal standard and cotinine signals coincided, the ion suppression present was corrected when concentrations were calculated. Additionally, cotinine (10 µg/L) was infused at a constant rate into the mass spectrometer, and extracts from 20 different cotinine-free serum samples were injected. Signal decreased by <10% when serum samples were injected.

The total time to complete this assay is substantially shorter than for other methods that require extensive extractions before assay (11, 13). The simple extraction into methylene chloride and a 3-min assay time combine to allow 100 samples to be analyzed in <8 h. Additionally, the 50-µL sample size makes this an ideal assay for use in large research studies or epidemiologic surveys where multiple analytes and limited sample volumes are common.

The described assay is simple, sensitive, precise, and requires only a small serum sample volume. Without the need for a HPLC column, analysis time is rapid (3 min), and is sensitivity suitable for the qualitative detection of recent passive nicotine exposure. We conclude that this method is ideally suited for analysis of cotinine in passive and active smokers for both clinical and research purposes.

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References


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The enzyme renin (Mw ~40 000) is released in an active form from the renal juxtaglomerular cells in response to physiologic factors, including sodium depletion, decreased blood volume and blood pressure, and β-adrenergic stimulation (1). Although several local angiotensin II-generating systems exist within various tissues (including the heart, brain, and adrenal glands), the concentration of active renin in plasma depends on the rate of renin secretion from the kidneys (2). Renin catalyzes the formation of angiotensin I (AngI) by cleavage of the renin substrate called angiotensinogen. Plasma renin is therefore the initiator of the renin-angiotensin-aldosterone system, which has an important role in the homeostasis of water and electrolyte balance and in the regulation of arterial pressure.

In most studies, circulating renin has been estimated by assays of plasma renin activity (PRA). PRA is measured by generating AngI from endogenous angiotensinogen, followed by measurement by RIA of the generated AngI. Although PRA measurement is convenient for estimating the biological activity of the renin system, it may not necessarily reflect the real concentration of active renin. The concentration of substrate rarely affects the PRA result, but exceptions do occur (3). More importantly, PRA depends not only on renin, but also on factors that influence the renin–substrate interaction.

An additional difficulty occurs in measuring low con-