Validation of Self-Reported Smoking Status Using Saliva Cotinine: A Rapid Semiquantitative Dipstick Method

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Abstract

Purpose: **This study evaluated the performance characteristics of a novel rapid method for verifying smoking status in individuals by measurement of cotinine, the primary metabolite of nicotine, in saliva samples using an immunochromatographic strip in a ''dipstick'' format compared with liquid chromatography/mass spectrometry (LC/MS).**
Materials and Methods: **A prospective comparison was made of smoking status as determined by measurement of cotinine in urine by LC/MS (the gold standard) and in saliva using a semiquantitative dipstick assay that uses cotinine-specific monoclonal antibodies attached to gold particles and a series of avidity traps to measure cotinine levels (saliva NicAlert®). One hundred seventy-two individuals from a family practice/general medical setting agreed to participate after informed consent and institutional review board approval. Saliva NicAlert® tests were done by untrained operators who followed written directions.**
Results: **Comparison of smoking status as determined by urine cotinine measurement by LC/MS (50 ng/mL cutoff) with the saliva strip test results, averaged over the two operators, indicated that the saliva test strip results had a sensitivity of 99% and a specificity of 96%. Saliva NicAlert® also identified four smokers who reported being nonsmokers but were confirmed to be smokers by LC/MS.**
Conclusions: **The saliva NicAlert® assay seems to be a valid, highly sensitive, and specific method for validating self-reported smoking status and may have clinical applications in selected medical settings.** (Cancer Epidemiol Biomarkers Prev 2007;16(9):1858–62)

Introduction

The use of tobacco products in general and cigarette smoking in particular is a major public health problem and is the leading preventable cause of disease in our society today. Tobacco use has been identified as a risk factor for cardiovascular diseases, lung and other cancers, chronic respiratory diseases, stroke, and complications of pregnancy (1-4). Annually, an estimated 438,000 people in the United States (5) and 4.9 million people worldwide (6) die prematurely as a result of tobacco use. Another 50,000 die each year as a result of exposure to second hand smoke [environmental tobacco smoke (ETS)]. Despite the public awareness of the health risks tobacco use poses, ~21.6% (45.4 million) adults in the United States currently smoke cigarettes (7). The annual health care costs attributable to cigarette smoking in the United States have been estimated at $75.5 billion and the attributed annual productivity losses at $92 billion (5).

Reducing tobacco use and dependence has been identified as a key strategy in reducing the significant long-term health effects and associated economic costs of tobacco use. Clinical practice guidelines recommend the consistent identification and documentation of tobacco users in the health care setting as the first step in clinical interventions to counsel and treat tobacco users (8-11), and it has been recommended that determining smoking status should be a vital sign (8, 12-14). Self-reported smoking status helps to identify a significant percentage of smokers but does not identify all smokers. Data suggests that a percentage of patients ranging from 1.4% in broadly based epidemiologic studies to as high as 35% in populations where smoking is a known risk factor, such as patients with respiratory disease (18%), cancer patients (20%), and pregnant women (35%) will self-report inaccurately due to a variety of factors (such as misunderstanding, intentional deception, embarrassment, denial, shame, etc.; refs. 15-20).

Measurement of cotinine, a primary metabolite of nicotine that has a half-life of 16 to 18 h and that can be detected in urine, saliva, or serum, provides a reliable means of determining smoking status and other tobacco product use or exposure over a period of 2 to 3 days (20-22). For smokers, another method of determining tobacco use is expired carbon monoxide. A relatively short half-life of 4 h limits the reliability and accuracy of detecting smokers using expired carbon monoxide testing, and carbon monoxide testing is unable to detect the use of smokeless tobacco (chew, dip, or snuff products). At this time, cotinine measurement has not been routinely used in clinical settings mainly due to the time involved, the methods required to collect the sample, and the cost

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and inconvenience of sending samples to laboratories. However, the validation of reported tobacco use status may have significant clinical importance in cessation clinics, research settings, pulmonary and pediatric clinics, or transplant candidate selection and may have an effect on the determination of insurance premiums.

This report evaluates a new rapid method for measuring cotinine levels using saliva samples to verify smoking status in individuals in a clinical setting, such as a family practice or other general medicine setting. An immunochromatographic test strip in ''dipstick'' format (saliva NicAlert®) was used to measure cotinine levels in saliva samples. The sensitivity and specificity of the urine dipstick NicAlert® method was previously independently validated by several groups with urine NicAlert® results being found to compare favorably to the ELISA laboratory method and to the mass spectrometry method (23-25). The ease and convenience of providing a saliva sample may make a saliva-based test more preferable in a clinical setting than a urine-based test.

In this study, a prospective comparison was made between self-reports of smoking status and a semiquantitative, enzyme-linked, immunosorbent assay-based method (saliva NicAlert®) testing for the presence of cotinine. Results were validated by urine liquid chromatography/mass spectrometry (LC/MS) by an independent reference laboratory.

Materials and Methods

Subjects. One hundred seventy-two individuals participated from a family practice/general medical setting after informed consent and institutional review board approval. Subjects were primary care outpatients at two different sites. Five patients (2.9%) were excluded from the final analysis: in three (of 172) cases, there was an inadequate amount of saliva provided by the subject, and in two (of 172) cases the operator was unable to report a reading from the strip (presumed to be due to technique or mishandling). Of the analyzed group (n = 167), 36.5% (n = 61) were men and 62.3% (n = 104) were women; for two subjects, sex was not recorded. The mean age of subjects was 42.1 years with the youngest subject being 6 years old and the oldest is 80 years old. The overall prevalence of current self-reported smoking in the group was 46.1% (n = 77). The characteristics of the study population are summarized in Table 1.

Materials and Methods. After informed consent was obtained, each participant filled out a standard checklist form, which included information on age, sex, current medications and dosage, self-reported tobacco products usage (cigarettes, pipes, cigars, chewing tobacco, or snuff, brand and type of cigarette, and quantity of tobacco product used in the previous 3 days), and self-reported ETS exposure (presence or absence of smokers in the home, in the work environment, or in social settings and number of hours in each of the above in the past 3 days). Participants were asked about their current smoking history, including any smoking in the last week, the number of cigarettes typically smoked per day, and the extent of second-hand smoke exposure.

Participants were asked to provide saliva and urine samples. The saliva sample was witnessed. Urine samples were collected using chain of custody methods, transferred to a separate tube, then frozen (−80°C) for blinded confirmation with LC/MS. These samples were evaluated at an independent reference laboratory (National Medical Services, Willow Grove, PA) using a modification of the method of Moyer et al. (26) on a Waters Micro Quattro with a limit of detection of 1 ng/mL.

Cotinine Analyses. Cotinine is a widely accepted biomarker for tobacco product use and exposure. Cotinine has a longer half-life (~20 h) than nicotine (~2 h) and can be measured in biological fluids, such as saliva, plasma, and urine. The NicAlert® test (Nymox Corporation) is a semiquantitative method that uses a dipstick to measure the level of cotinine in a sample of saliva or urine based on a colorimetric immunoassay reaction. NicAlert® also detects other nicotine-cotinine derivatives, such as 3-hydroxycotinine.

Saliva samples were collected and tested with NicAlert®. Each NicAlert® strip was independently read on site according to the manufacturer's written instructions by two physician assistants who were untrained (Tennessee site) and two untrained (medical assistant and licensed practical nurse) office personnel (West Virginia site) and the results recorded. The test strip displays seven ''zones'' (Fig. 1), with each zone representing a range of levels of cotinine/smoking [e.g. zone 0 (0–10 ng/mL, a nonsmoker) to zone 6 (>1000 ng/mL, a heavy smoker)]. The results read were recorded as values from 0 to 6 (see Fig. 1).

The urine samples were frozen (−80°C) for blinded confirmation with LC/MS.

Table 1. Characteristics of study population

		All	Males	Females
		N = 167*	n = 61	n = 104
Mean age (y)		42.1	41.6	42.3
Range of ages (y)		6-80	6-68	12-80
Self-reported smoking status	Smoker	77 (46.1%)	25 (41.0%)	51 (49.0%)
	Nonsmoker	90 (53.9%)	36 (59.0%)	53 (51.0%)

*Sex of two subjects was not noted.

Results

The saliva NicAlert® cutoff for smoker versus nonsmoker is 1. Results determined by NicAlert® (≥1) indicates that the subject is a smoker or has significant exposure to ETS. Based on a comparison between the reported results for the two untrained operators at each site, the overall agreement in determining smoking status was 96.3% at site 1 (κ 0.92, 95% confidence interval 0.71-1.00, $P < 0.0001$) and 98.7% at site 2 (κ 0.97, 95% confidence interval 0.75-1.00, $P < 0.0001$). The data in Table 2 display the sensitivity of saliva NicAlert® to detect smokers as determined by LC/MS based on a 50 ng/mL cutoff was 100% for three of the four operators and 96.7% for the fourth operator. The specificity of saliva NicAlert® to detect nonsmokers as determined by LC/MS ranged from 100% for one operator (no false positives) to 91.8%

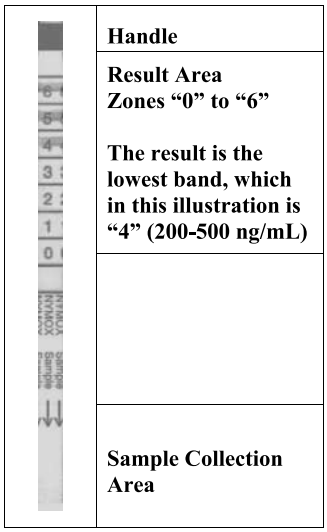

Figure 1. The NicAlert® strip.

(four false positives). One of the false positives was a teenage male with a cotinine level as determined by LC/MS near the cutoff (30.6 ng/mL) and reported ETS exposure in the home; another two reported significant ETS exposure at work.

The LC/MS readings according to self-reported smoking status are summarized in Table 3. There were no reported smokers or nonsmokers within 20% (±10 ng/mL) of the 50 ng/mL cutoff for urine LC/MS used in the study. One reported smoker of one to two pipes and cigars per day had an LC/MS reading of 8.8 ng/mL; all other reported smokers or users had LC/MS readings of >150 ng/mL. Eight subjects reported using chewing tobacco alone; one reported using both chewing tobacco and smoking cigarettes. Of the reported smokers, 3 reported light use (half pack a day or pipes and cigars), 19 reported smoking half pack of cigarettes a day, 36 reported one pack, 8 reported one and a half packs, and 3 reported two packs. Three reported nonsmokers had LC/MS readings of >200 ng/mL, indicating likely deception. One reported nonsmoker reporting ETS exposure of 5 to 8 h at work and 9 to 12 h at home had a urine LC/MS reading of 86.1 ng/mL. A second reported nonsmoker with reported ETS exposure of 9 to 12 h at home had a urine LC/MS reading of 30.6 ng/mL; all other reported nonsmokers (79 of 84 or 94%) had urine LC/MS values of <20 ng/mL, with 74 of 84 or 88% having urine LC/MS values of <10 ng/mL.

Reference laboratory testing of urine cotinine by LC/MS, using a cutoff of 50 ng/mL, identified 98.7% (76 of 77) of the self-reported smokers. The one (of 77) false negative was an individual who self-reported smoking one to two pipes/cigars a day. The saliva NicAlert® results also reported this individual as a nonsmoker. LC/MS identified four reported nonsmokers as smokers based on the urine cotinine levels determined by LC/MS. Saliva NicAlert® also was able to identify the same four self-reported nonsmokers as smokers based on their NicAlert® readings. Three of these individuals identified were clearly ''deceivers,'' with cotinine values ranging from 202 to 1051 ng/mL; the fourth was a likely deceiver with a cotinine value of 86 ng/mL but also reported significant ETS exposure at work, home, and socially. No LC/MS results were reported for six subjects, and the individual operators were not able to provide results for seven strips (7 of 322 or 2.1%).

Discussion

The use of an easy to obtain, reliable, highly sensitive, and specific assay for tobacco use status that can be done by untrained personnel at the point of care should have a

Table 2. LC/MS determination of smoking status (50 ng/mL cutoff) versus urine NicAlert® determination for the two operators at each site

LC-MS	Site 1			
	Operator 1		Operator 2	
	Nonsmoker (NicAlert® = 0)	Smoker (NicAlert® ≥ 1)	Nonsmoker (NicAlert® = 0)	Smoker (NicAlert® ≥ 1)
Nonsmoker (<50 ng/mL)	47	2	45	4
Smoker (≥50 ng/mL)	0	32	1	29
Sensitivity	100%		96.7%	
Specificity	95.9%		91.8%	

LC-MS	Site 2			
	Operator 1		Operator 2	
	Nonsmoker (NicAlert® = 0)	Smoker (NicAlert® ≥ 1)	Nonsmoker (NicAlert® = 0)	Smoker (NicAlert® ≥ 1)
Nonsmoker (<50 ng/mL)	32	0	31	1
Smoker (≥50 ng/mL)	0	47	0	44
Sensitivity	100%		100%	
Specificity	100%		96.7%	

NOTE: No LC/MS results were reported for six subjects, and the individual operators were not able to provide results for seven (of 322) strips (2.1%).

Table 3. Summary of LC/MS values in reported smokers versus reported nonsmokers

Self-reported smoking status		LC/MS, ng/mL
Reported nonsmokers (n = 84)	Mean*	29.3
	SD*	149.6
	Minimum Reading	BLQ (≤1)
	Maximum Reading	1051.1
Reported smokers/ users (n = 77)	Mean*	1851.9
	SD*	1537.8
	Minimum Reading	8.8
	Maximum Reading	ULOQ (>8000)
Total subjects (N = 161)		

Abbrevations: ULOQ, upper limit of quantitation; BLQ, below limit of quantitation.
*Reported below limit of quantitations treated as a reading of 1 ng/mL.

significant role in many clinical and research settings when the accurate assessment of tobacco use or exposure is required. The present study has the limitations that it was undertaken in a family medicine/general practice population and did not include significant numbers from obstetric, infant, or geriatric populations. However, the NicAlert® assay would be expected to perform as reliably with any of these groups. Further research to validate this theory may be required. The percentage of reported smokers in the study (46%) was greater than that reported for the general population in the two states where the study was conducted (25.7%, Tennessee 2005; 27.4%, West Virginia 2005) (27).

The very high sensitivity of the NicAlert® assay (100% for three of the four operators; 96.7% for the fourth operator) allows for rapid, consistent identification and documentation of tobacco use in the health care setting and provides clinical information to guide counseling and treatment of tobacco use status. In this study, the saliva NicAlert® test was able to correctly identify the four deceivers (users reporting no use), which was confirmed by LC/MS. The low false positive rate (high specificity) of the NicAlert® assay provides an objective determination of tobacco use or exposure and can aid in the selection of interventions to those who use tobacco or may have significant exposure to tobacco. Three of the four individuals with false positive results reported a high degree of ETS exposure, a clinically significant finding in itself, given the health effects of ETS exposure, particularly for patients with respiratory conditions, such as asthma, chronic obstructive pulmonary disease, and cardiovascular disease (22).

The saliva dipstick methodology has potential application in a clinical setting to objectively validate the assessment of smoking status or exposure or smokeless tobacco use and to help guide interventions, such as counseling for cessation, detection of relapse, or the need to reduce environmental tobacco exposure. A semiquantitative saliva assay has the advantage in the clinic of being able to provide immediate feedback without the need for interruption or leaving the room and returning later (e.g. urine assay) or mailing to a reference laboratory (e.g. blood or urine assays). The more immediate test result could improve the value from counseling and improve patient compliance with tobacco avoidance during the clinical encounter. The method cannot, however, distinguish between smokers and ex-smokers using nicotine replacement therapy.

In clinical and research settings, an accurate, easily done, sensitive, and reliable determination of self-reported smoking status can be vital to plan appropriate disease management and risk reduction strategies, particularly for diseases or conditions exacerbated by smoking or other tobacco use, such as chronic obstructive pulmonary disease, asthma, lung cancer, pregnancy, or coronary artery disease. Smoking cessation can be encouraged for patients with coronary artery disease before bypass grafting to reduce their risks of myocardial infarction and perioperative surgical complications. In some cases, the awareness of tobacco use could direct a more accurate approach to risk assessment or reduction. For example, accurate calculation of 10-year Framingham risk scores depends on smoking status in those with hyperlipidemia. A positive saliva NicAlert® result may help identify patients for pulmonary function testing in those patients who complain of shortness of breath or other chronic respiratory symptoms but for some reason may deny (or minimize) smoking or ETS exposure. The selection of patients who may be candidates for lung transplant or other types of transplant surgery may be improved.

From a research perspective, it is widely recognized that studies evaluating disease outcomes related to smoking and cessation rates should incorporate the most sensitive and specific objective measurement for confirming self-report. Misclassification may affect efficacy data in clinical trials. Calculations of smoking-related risk in both smokers and nonsmokers may be affected, depending on study size. The use of the dipstick saliva device simplifies the measurement procedure and may be helpful in research studies where smoking status or ETS exposure is an inclusion or exclusion criterion or a variable to be controlled for in the research analysis. Furthermore, unlike a urine test, a saliva sample can easily be witnessed.

Stopping teenagers from initiating or continuing to smoke remains a high public health priority. The validation of tobacco use status using easily obtainable saliva samples may also be appropriate for pediatric or adolescent patients where early detection of tobacco use may result in early intervention, improved health, and a reduction in acute or chronic illness, such as asthma. Although the majority of chronic diseases that result from the effects of tobacco use occur later in life, most current smokers start smoking in their teens, and tobacco is associated with other high risk behavior, including illegal drug use (28).

Although it is feasible that this saliva method could be used to identify minors exposed to ETS, it would mainly be applicable to individuals with heavy exposure to ETS. The urine-based strip is more practical for the detection of ETS exposure because of the higher levels of cotinine in urine compared with saliva in the same individual.

In summary, there is a need in some clinical and research settings for a simple, on-site test to verify tobacco use status or exposure to tobacco smoke. The saliva NicAlert® test was able to distinguish tobacco users from nonusers in a clinical setting with high degree of accuracy using LC/MS as the reference standard. The

saliva dipstick has many potential applications in clinical settings as part of the routine assessment of smoking status as a vital sign and to help guide clinical interventions and risk management. In research settings, this methodology for tobacco use or exposure could improve patient selection, data collection, and analysis. For insurance companies, tobacco use may influence premium rates. However, the attraction of this and other cotinine detection or smoking status tests will depend on test costs, methods and amounts of reimbursement for performing the tests, and clinicians' experience of the utility and value of such testing in a clinical setting.

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