

**BRIEF REPORT****AN EVALUATION OF NICCHECK I®: A DIPSTICK METHOD FOR ANALYZING NICOTINE AND ITS METABOLITES**

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Abstract— This study examined the reliability of a test strip for assessing urinary nicotine and its metabolites. Urine samples from smokers were tested by two independent raters using the NicCheck I® test strips. Each rater compared the test strip color to a five-color chart. Correlations between the rater's codings and between the coding and other measures of nicotine consumption were examined. Significant correlations were found between the two independent ratings of the test strip color and between the codings and other measures of nicotine consumption. This preliminary evaluation suggests that NicCheck I® is sensitive to differences in nicotine consumption. © 1998 Elsevier Science Ltd

Biochemical markers of smoking are more accurate measures of nicotine intake than self-reported use of cigarettes (Perez-Stable, Marin, Marin, & Benowitz, 1992; Wagenknect, Burke, Perkins, Haley, & Friedman, 1992). Cotinine, the major metabolite of nicotine, which can be measured in blood, saliva, or urine, is a commonly used biochemical indicator of smoking status (Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987). The elimination half-life of cotinine is approximately 20 h, and a relatively constant concentration of cotinine is maintained in the body during the smoking day. Both the sensitivity and specificity of cotinine as a marker of cigarette smoking are greater than 95% (Benowitz, 1983; Jarvis et al., 1987). Typically, cotinine levels are assessed using gas chromatography or radioimmunoassay, which are costly procedures for clinical applications.

Because breath carbon monoxide (CO) is cheaper and easier to administer than cotinine assessments, CO levels are the biochemical marker most frequently employed in clinical applications. CO has acceptable sensitivity and specificity (about 90%) (Benowitz, 1983; Jarvis et al., 1987) and provides immediate feedback about smoking status. However, CO has a short half-life (about 4 h) (Jarvis et al., 1987). Consequently, the accuracy of CO for determining smoking status is affected by both the time of day of the assessment and the time since the last cigarette was smoked. It is possible for smokers to alter their smoking behavior in order to appear abstinent (Stitzer & Bigelow, 1983). What is needed is a cost-effective biochemical assessment procedure that provides immediate feedback about smoking status that is not sensitive to modifications in smoking behavior.

This work was supported by a grant from Marion Marell Dow, no. 432370. The authors wish to thank DynaGen, Inc., for providing the urinary test strips used in the study.

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NicCheck I[®] (DynaGen, Inc., Cambridge, MA) is a urine test strip that determines the urinary concentration of nicotine and its metabolites based on a colorimetric reaction. It was designed to provide a rapid test of smoking status for use in a clinical setting. The test strip is dipped into participant's urine and changes color (varying shades of pink) in the presence of nicotine and its metabolites. The level of nicotine and its metabolites is determined by matching the test strip with a color chart provided by the manufacturer. For the test strip to be useful in clinical applications, comparisons between the test strip and the color chart must be consistent across different raters.

The purpose of this preliminary investigation was to provide information about the reliability of test strip codings and the relationship between test strip codings and other measures of nicotine consumption: CO, cigarettes per day (CPD), and urinary cotinine and nicotine. In addition, a secondary goal was to assess whether a three- or five-color coding scheme would provide the greatest reliability.

METHODS

Sixty-seven Hispanic smokers participating in a clinical trial of the efficacy of the nicotine patch in smoking cessation participated. On average, participants were 40.8 years old ($SD = 8.7$), had been smoking for 22.5 years ($SD = 9.6$), and smoked 18.6 ($SD = 9.0$) cigarettes per day; 54% were female. The study was approved by the Human Subjects Committee at the University of Arizona, and informed consent was obtained.

A pre-quit urine sample (approximately 60 ml) was collected and stored at -20°C . The samples were later thawed to room temperature, and 0.5–1.0-ml samples were dispensed into test tubes. NicCheck I[®] test strips were inserted into the test tubes with forceps and were tested independently by two raters 25 to 30 min later. Each rater independently compared the test strip color to a color chart that ranged from white to dark pink. The chart contained three primary color categories and two intermediate colors, all gradations of pink. Each color category was assigned a corresponding numerical code. If the two independent ratings were not the same, the raters later made a consensus rating. The five-level ratings were converted to a three-level rating by combining the two intermediate colors with two of the primary color categories.

RESULTS

Interrater reliability of codings was assessed by calculating the concordance rate between the codings. The raters agreed on 77.6% (52/67) of the samples for the five-level coding and on 86.6% (58/67) of the samples for the three-level codings. There were no systematic differences between the codings of the two raters (both $t(66) < 1$). The reproducibility of these results was evaluated using the Kappa statistic. The reproducibility was marginal (Kappa = .4) for the five-level coding and was good (Kappa = .7) for the three-level coding. Also, the codings of the two raters for both the three- ($r = .78, p < .001$) and the five-level ratings ($r = .87, p < .001$) were significantly correlated, indicating a high level of agreement between the two raters.

The relationship between the color codings and other measures of nicotine consumption was examined. Because the raters did not agree 100% of the time on their coding of the test strips, the consensus ratings were used in these analyses. Table 1 presents the mean CO level, CPD, and mean nicotine and cotinine levels for each NicCheck I[®] category and correlations among each of these measures and the three- and

Table 1. Measures of nicotine consumption as a function of NicCheck I[®] categories

NicCheck category	Nicotine consumption measure			
	Breath CO	Cigs/day	Cotinine	Nicotine
Five-Color Coding				
1 (N = 11)	16.1 (6.8) ^a	14.8 (5.9)	540.6 (743.3)	566.4 (586.4)
2 (N = 16)	16.8 (7.9)	15.1 (4.9)	757.7 (308.1)	789.6 (666.5)
3 (N = 32)	21.2 (10.6)	19.5 (9.4)	1316.2 (1037.6)	2413.7 (2543.4)
4 (N = 8)	26.0 (6.7)	24.4 (11.7)	1698.5 (1345.6)	4201.9 (4417.7)
5 (N = 0)	N/A	N/A	N/A	N/A
Spearman <i>r</i>	.36*	.29*	.48*	.52*
Three-Color Coding				
1 (N = 11)	16.1 (6.8)	14.8 (5.9)	540.6 (743.3)	566.4 (586.4)
2 (N = 48)	19.9 (9.9)	18.0 (8.3)	1130.0 (900.6)	1872.3 (2230.9)
3 (N = 8)	26.0 (6.7)	24.4 (11.7)	1698.5 (1345.6)	4201.9 (4417.7)
Spearman <i>r</i>	.32*	.23*	.45*	.42*

^aValues mean \pm SD.

* $p < .05$.

five-level consensus ratings, respectively. Correlations between the NicCheck I[®] three- and five-level codings were all in the expected direction, with darker NicCheck I[®] color being positively and significantly correlated with higher breath CO, CPD, and urinary cotinine and nicotine. It should be noted that 11 of the 67 test strip evaluations resulted in a false-negative reading.

DISCUSSION

This preliminary and limited evaluation of NicCheck I[®] suggests that it is sensitive to differences in nicotine consumption; however, continued refinement and evaluation of this technology is needed. For example, it is not clear whether the false-negatives were influenced by several freezings and thawings of the urine samples, inaccuracy of the technology, or for some other reasons. Nonetheless, the overall results of this preliminary investigation are quite promising, and they clearly warrant further evaluation of this technology. Between the time this study was conducted and now, manufacture of the strips has changed, and the current investigators have recently retested the strips as part of a trial for FDA review.

Correlations between the test strip codings and other measures of nicotine consumption were significant but low. Measures of consumption are not perfect indicators of exposure owing to individual variations in puffing techniques, nicotine metabolism, etc., and any of these variations would serve to attenuate the overall correlation. Correlations were positive, indicating the darker NicCheck I[®] colors were associated with higher values of the indicators of nicotine exposure. This finding suggests that NicCheck I[®] is sensitive to differences in nicotine intake and may have the potential to provide a quantitative index of nicotine and its metabolites.

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