MOSSMAN ASSOCIATES

GC ANALYSIS OF 3-HYDROXYCOTININE CONTENT IN URINE AND DETERMINATION OF CORRELATION WITH NicCheck I RESULTS

NOTE:

The following is a summary of the comparison between GC results and our NicCheck I test. While one cannot quite use NicCheck for quantitation because of variability in how people interpret and read colors, there is a very good correlation with GC results in identifying active consumers of nicotine. In fact, GC is somewhat restrictive because one only gets the cotinine results from GC (we have done 3-hydroxycotinine as well because cotinine is quickly converted by metabolism to 3-hydroxycotinine). Cotinine values by themselves do not provide a **COMPLETE** picture of nicotine consumption. Because NicCheck measures ALL metabolites of nicotine, the color on NicCheck provides a COMPLETE assessment of nicotine consumption. While the color contribution on NicCheck by the various metabolites varies, one still gets an overall picture with NicCheck as compared to the GC methodology.

SUMMARY

As mentioned in the original 510(k) submission, frozen aliquots of urine from the clinical samples were sent to Dr. Benowitz's laboratory at the University of San Francisco for analysis of cotinine and nicotine content in the urine by gas chromatography (GC). Also, analyses of the NicCheck I positive and GC positive (urine cotinine values > 200 ng/mL by gas chromatography) urine specimens for 3-hydroxycotinine content was subsequently undertaken because of the improved correlation between the NicCheck® I and GC results observed when nicotine plus cotinine values were included in the analysis. Results demonstrate that when 3-hydroxycotinine values are included in the correlation analysis, the occurrence of a "+" (low) NicCheck I color reading correctly identified subject samples containing <12,500 ng/mL of nicotine plus cotinine plus 3-hydroxycotinine 90% of the time, and the occurrence of a "++" (high) NicCheck I color reading correctly identified subject samples containing 12,500 ng/mL of nicotine plus cotinine plus 3-hydroxycotinine 82% of the time. This is an improvement over the 73% and 76% respective correlation observed when cotinine alone was used as a comparator, and the 78% and 80% respective correlation observed when only cotinine plus nicotine were used as the comparator. Therefore, it can be concluded that if other metabolites were also to be included in the correlation analysis, the overall correlation of the NicCheck I test in classifying nicotine consumers as "low" versus high based on *nicotine* and *total* metabolites present in the urine, would approach 100%.

In view of the correlation between the intensity of color present on the NicCheck test strip and the total amount of nicotine and/or metabolites present in the urine which are detectable by GC, NicCheck I can be used as a practical means of differentiating low versus high consumption of nicotine.

Clinical Data

The clinical evaluation of the NicCheck I test was performed at 3 clinical sites: LeeCoast Research Center, Ft. Myers, FL (Dr. B. M. Phillips, Principal Investigator); University of Michigan, Department of Psychiatry and Behavioral Medicine (Dr. C.J. Pomerleau, Principal Investigator); and Arizona Nicotine and Tobacco Research Program, Tucson, AZ (Dr. S.J. Leischow, Principal Investigator). The studies were performed under Institutional Review Board approval and with informed consent. Each site tested urine specimens from 50 nonsmokers and from 78 to 91 smokers. For the smokers, approximately 50 smokers at each site were categorized as "high" based on carbon monoxide (CO) in exhaled air of greater than 20 ppm and the remaining smokers at each site were categorized as "low" based on CO in exhaled air of 11-20 ppm. Individuals consuming nicotine by modes other than smoking of cigarettes were excluded from the study. This was based on the fact that individuals smoking pipes and cigars may not inhale to the same extent that cigarette smokers do, and therefore, the level of CO in their expired air would not accurately represent their smoking status (Dr. Neal Benowitz, personal communication). This was also demonstrated clearly by Wald et al. in two separate publications in 1981 and 1984. Copies of these publications were provided in the original 510(k) submission. In a personal communication with Dr. Elbert D. Glover of West Virginia, he stated that an individual who starts consuming nicotine only as a cigar smoker probably inhales less, when compared to an individual who starts consuming nicotine as a cigarette smoker and then switches to cigar smoking. These factors introduce considerable variability in extent of inhalation, and therefore in the level of CO in exhaled air. Consumers of nicotine by means of chewing tobacco or snuff were studied as part of a separate protocol since CO monitoring in such individuals would not yield any information on their tobacco consumption. Results will be provided to the FDA as soon as they are available.

All subjects were asked to complete a questionnaire providing information on demographics, smoking habits, exposure to secondhand smoke, exposure to environmental sources of CO, consumption of nicotine by modes other than smoking, and concomitant medications, if any. For each subject, the CO in exhaled air was measured by Bedfont Scientific Ltd.'s EC 50 Smokerlyzer; this information was used to categorize the subjects. A urine sample was obtained from each subject. All testing was performed by a maximum of two individuals at each site. The testing was performed blind. The NicCheck I test was performed on this specimen, and two aliquots were stored frozen at -20 °C. At the end of the study, that of the EC 50 Smokerlyzer.

Of the total of 150 nonsmokers classified as such on the basis of the measurement

of CO in exhaled air as determined by the EC 50 Smokerlyzer, there were 4 false positive results by the NicCheck I test for an overall specificity of 97.3% with a 95% confidence interval of 93.3% to 99.3%. Of the total of 249 smokers classified on the basis of the measurement of CO in exhaled air as determined by the EC50 Smokerlyzer, 241 were identified as smokers by the NicCheck I test for an overall sensitivity of 96.8% with a 95% confidence interval of 93.8% to 98.6%. Results are demonstrated in Table 1.

Of the 249 smokers and 150 nonsmokers tested with the NicCheck I test, a positive predictive value of 98.4% and a negative predictive value of 94.8% were demonstrated.

With regard to cotinine determination by gas chromatography (GC), the relative sensitivity and relative specificity of the NicCheck I test compared to cotinine values as determined by GC is provided in Table 2. The overall specificity was 97.4% with a 95% confidence interval of 93.4% to 99.3%. The overall sensitivity was 97.6% with a 95% confidence interval of 94.8% to 99.1%. As can be seen, these numbers are comparable to the relative sensitivity and specificity obtained upon comparison of the NicCheck I test with the CO results.

Of the 247 smokers and 152 nonsmokers tested with the NicCheck I test and compared to cotinine GC analysis, a positive predictive value of 98.4% and a negative predictive value of 96.1% were demonstrated.

There was no significant correlation between the classification of smokers into low" versus "high" categories based on the CO results versus the classification as "low" versus "high" by the NicCheck I test. Since the half life of CO in exhaled air is 4-6 hours, and the CO reading is strongly influenced by when the last cigarette is smoked, it is not surprising that the classification of "low" versus "high" smokers based on CO levels does not correlate with a classification based on the detection of nicotine and/or its metabolites.

It should be noted that neither CO levels nor NicCheck I results as "low" or "high" correlate well with the self reported number of cigarettes smoked. Again, this is not unexpected since it is a well accepted fact that individuals smoke different kinds of cigarettes (containing various amounts of nicotine), inhale differently, and the rate of nicotine metabolism also differs from individual to individual. The coefficient of correlation between number of cigarettes smoked and GC cotinine values, an established method for assessment of nicotine dependence, is only ~ 0.45 (Benowitz et al, 1983). Additionally, self reports of nicotine consumption are not considered reliable, although they may be more accurate than normal in this study since there was no physician intervention and therefore no reason to misreport one's smoking habit. All of the above factors contribute to a less than perfect correlation between numbers of cigarettes smoked and the classification of smokers as "low" versus "high" consumers of nicotine.

The data among those positive by NicCheck I and positive by GC for urine cotinine (employing a cutoff value of 200 ng/mL) were further analyzed to determine

whether the NicCheck I test might provide a differentiation between levels of nicotine consumption. The data are presented in Table 3 comparing the results, employing various cotinine levels as the cutoff for discrimination of "+" (low) or "++" (high) by the NicCheck I test. These data show that the occurrence of a "+" NicCheck I color reading correctly identified subject samples containing <1500 ng/mL of cotinine 73% of the time and the occurrence of a "++" NicCheck I color reading correctly identified subject samples containing 1500 ng/mL of cotinine 76% of the time. It should be noted 1500 ng/mL of urine cotinine is approximately equivalent to 250 ng/mL of plasma cotinine (urine cotinine values are 5-6 fold higher than plasma cotinine values), and it is well accepted that 250 ng/mL of plasma cotinine may be used as a cutoff value for the differentiation between low dependent and high dependent smokers (Paoletti et al.,1996; Sachs, 1995). Copies of these reference papers were provided in the original 510(k) submission.

When the same 2 X 2 table is constructed for a comparison between NicCheck I positive results and the sum of nicotine and cotinine values in urine as determined by GC (Table 4), it was found that and the occurrence of a "+" NicCheck I color reading correctly identified subject samples containing < 3500 ng/mL of nicotine plus cotinine 78% of the time and the occurrence of a "++" NicCheck I color reading correctly identified subject samples containing 3500 ng/mL of nicotine plus cotinine 80% of the time. This is not unexpected since the NicCheck I test detects nicotine as well as nicotine metabolites.

Nicotine is metabolized into cotinine, its primary metabolite. However, cotinine is further metabolized into predominantly 3-hydroxycotinine, which comprises approximately 60% of the total metabolite pool (Benowitz et al, 1990 - a copy of this paper can be found in Appendix A). Based on research conducted at DynaGen, it is known that the NicCheck I test also detects 3-hydroxycotinine. It is therefore logical to expect that when the sum of nicotine, cotinine and 3-hydroxycotinine is included in the correlation analysis, the overall classification by the NicCheck I test into a "low" versus "high" level of nicotine consumption would be even more comparable than the 78% and 80% correlation respectively obtained when only the sum of nicotine and cotinine is taken into account. The determination of the concentration of 3-hydroxycotinine in the NicCheck I positive urine samples with GC urine cotinine values >200 ng/mL was therefore undertaken. The testing was again conducted at the laboratory of Dr. Neal Benowitz at the University of California in San Francisco.

A 2 X 2 table was plotted for comparison between NicCheck I positive results classified as "low" or "high" versus the sum of urine cotinine plus nicotine plus 3-hydroxycotinine, as determined by GC (Table 5). It was found that the occurrence of a "+" NicCheck I color reading correctly identified subject samples containing <12,500 ng/mL of nicotine plus cotinine plus 3-hydroxycotinine 90% of the time, and the occurrence of a "++" NicCheck I color reading correctly identified subject samples containing 12,500 ng/mL of nicotine plus cotinine plus 3-hydroxycotinine 82% of the time. This is an improvement over the 73% and 76% respective correlation observed

when cotinine alone was used as a comparator, and the 78% and 80% respective correlation observed when cotinine plus nicotine were used as the comparator. This has been depicted in Figure 1.

Furthermore, since the NicCheck I test detects other nicotine metabolites in addition to cotinine and 3-hydroxycotinine, and also the fact that nicotine plus cotinine plus 3-hydroxycotinine constitute only 70-75% of the total nicotine metabolite pool in the urine, it can be assumed that the overall correlation of the NicCheck I test in classifying nicotine consumers as "low" versus "high", based on nicotine plus *total* metabolites present in the urine would approach 100% when the contribution of color provided in the NicCheck I reaction by the remaining untested nicotine metabolites is taken into consideration.

Therefore, in view of the correlation between the intensity of color present on the NicCheck test strip and the total amount of nicotine and/or metabolites present in the urine which are detectable by GC, NicCheck I can be used as a practical means of differentiating low versus high consumption of nicotine.

Table 1
NicCheck I results compared to CO

	Site			
	Arizona	Florida	Michigan	Overall
Relative Sensitivity	89/91= 97.8%	80/80 = 100%	72/78 = 92.3%	241/249 = 96.8 %
Relative Specificity	48/50 = 96%	49/50 = 98%	49/50 = 98%	146/150 = 97.3%

Table 2
NicCheck I results compared to cotinine by GC*

	Site			
	Arizona	Florida	Michigan	Overall
Relative Sensitivity	90/91 = 98.9%	79/79 = 100%	72/77 = 93.5%	241/247 = 97.6%
Relative Specificity	49/50 = 98%	49/51 = 96.1%	50/51 = 98%	148/152 = 97.4%

*Based on 200 ng/mL as the cutoff for smokers, using GC

Table 3
Distribution of NicCheck I Results vs. Urine Cotinine Analysis by Gas Chromatography (GC)

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine < 1000 ng/mL by GC	Urine Cotinine 1000 ng/mL by GC
NicCheck, +	71	104
NicCheck, ++	6	60

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine < 1500 ng/mL by GC	Urine Cotinine 1500 ng/mL by GC
NicCheck, +	127	48
NicCheck, ++	16	50

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine < 2000 ng/mL by GC	Urine Cotinine 2000 ng/mL by GC
NicCheck, +	150	25
NicCheck, ++	29	37

Table 4

Distribution of NicCheck I Results vs. Cotinine and Nicotine Analysis by GC

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine < 3000 ng/mL by GC	Urine Cotinine + Nicotine 3000 ng/mL by GC
NicCheck, +	123	53
NicCheck, ++	10	56

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine < 3500 ng/mL by GC	Urine Cotinine + Nicotine 3500 ng/mL by GC
NicCheck, +	138	38
NicCheck, ++	13	53

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine < 4000 ng/mL by GC	Urine Cotinine + Nicotine 4000 ng/mL by GC
NicCheck, +	147	29
NicCheck, ++	17	49

Table 5
Distribution of NicCheck I Results vs. Cotinine plus Nicotine plus 3-Hydroxycotinine Analysis by GC

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine + 3-hydroxycotinine < 11,500 ng/mL by GC	Urine Cotinine + Nicotine + 3-hydroxycotinine 11,500 ng/mL by GC
NicCheck, +	148	27
NicCheck, ++	11	55

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine + 3-hydroxycotinine < 12,500 ng/mL by GC	Urine Cotinine + Nicotine + 3-hydroxycotinine 12,500 ng/mL by GC
NicCheck, +	157	18
NicCheck, ++	12	54

Positive NicCheck Readings	Number of Subjects With	
	Urine Cotinine + Nicotine + 3-hydroxycotinine < 13,500 ng/mL by GC	Urine Cotinine + Nicotine + 3-hydroxycotinine 13,500 ng/mL by GC
NicCheck, +	160	15
NicCheck, ++	13	53