Letter

Dipstick Tests For Cotinine: Comment On the Article by Best et al.

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We have read the recent paper by Best, Green, Smith, and Perry (2010) and wish to point out deficiencies and inaccuracies in the report. The authors employed urine samples spiked with cotinine and analyzed by liquid chromatography/mass spectrometry (LC/MS) with the useful purpose to assess whether NicAlert and TobacAlert immunochromatographic dipsticks were suitable for use in detecting secondhand smoke (SHS) exposure. In fact, TobacAlert is indicated for use in the detection of SHS exposure at levels of ≥6 ng/ml urinary cotinine, and NicAlert is not indicated for SHS testing.

The substantial discrepancies between the expected cotinine-spiked urine samples values and the LC/MS results for the respective samples are troubling and render the study’s data uninterpretable. If the authors’ own preparations fail to match the LC/MS results, then either the samples or their LC/MS results or both are invalid. The authors write (p. 553) “After the LC/MS results for the first validation exercise were reported; we noted substantial differences between LC/MS-determined cotinine levels and our intended ‘spike’ levels. We suspect that the spiking process was not correctly performed. We therefore ran a second validation exercise. We reported the results of both exercises since the LC/MS-determined levels, not the ‘spiked’ levels, were considered to be the ‘gold standard.’”

The authors’ suspicion that the “spiking process” was responsible for these substantial discrepancies is not supported by evidence of any investigation ruling out other sources of such errors, such as handling and/or labeling errors. In fact, the data from the repeated second validation exercise also show substantial differences between LC/MS-determined cotinine levels and spiked values. For example, the 2A-spiked sample of 5 ng/ml had an LC/MS reading of 71.522 ng/ml, more than 14-fold higher than expected. One cannot assume that sample spiking preparation error was solely responsible for discrepancies in results when other equally or more plausible explanations are not ruled out. Examination of the reported dipstick readings shows that the majority of samples tested by the dipstick method accorded reasonably well with LC/MS levels, but some samples were extremely deviant. This is more consistent overall with handling and/or labeling errors.

The Supplemental Data available online provides further evidence of problems with the samples used in both validation attempts in the study. For example, the “E” samples were “unspiked controls.” Six of six TobacAlert strips used to test the 2Er and 3Er samples in the second validation attempt produced results ("0" readings indicating <10 ng/ml) that accorded 100% with both the spiked and the LC/MS values, while all three TobacAlert strips used to test the 1Er sample produced discordant elevated readings ("1" and "2" readings indicating <100 ng/ml). A similar pattern occurred with NicAlert testing of the same (1Er) sample where all three NicAlert strips used to test the 1Er sample again produced a discordant elevated reading ("2"). It is extremely improbable that this highly segregated abnormal pattern of discordant readings is due to random variations in dipstick quality or performance (two-sided Fisher Exact test for the TobacAlert 1Er sample, 9/9 false positive and 18/18 true negative, p < .0001). If it was a dipstick problem, the discordant readings would be expected to be more randomly found throughout the data and not segregated so sharply. The identical skewed pattern is readily discernible in the data for the TobacAlert first validation exercise "E" samples (p < .0001), TobacAlert first validation exercise "A" samples (p < .0001), NicAlert second validation exercises "C" samples (p < .0001), and others. This repeated and highly significant improbability clearly points to methodological problems with individual samples, the reporting of the LC/MS values or both.

The paper is in error where it states that “The manufacturer of NicAlert and TobacAlert tests makes no statement regarding cross-reactivity to these species and makes no statement about sample factors such as pH or specific gravity that affect results” and that “The manufacturer’s literature for the test strips makes no statement about urine characteristics that would affect test results.” The product inserts in fact summarize cross-reaction and interferences studies in sections respectively entitled Cross-Reactions and Interference Studies. The product inserts specifically address 3-OH cotinine cross-reactivity as follows: “3-OH cotinine is a known cross-reactant with cotinine in immunoassays. 3-OH cotinine was spiked into cotinine negative ("0" NicAlert) urine at the following concentrations: 50, 150, 250, 750, and 1,200 ng/ml. 3-OH cotinine showed a 12%-40% cross-reactivity with cotinine in the NicAlert assay.” The product inserts also contain extensive information outlining interference studies conducted including specifically pH: “The effects of other interfering substances and variables were examined in the NicAlert test. Ascorbic acid, pH, specific gravity, bacteria, protein, hemoglobin, bilirubin, and glucose were spiked into urine...”

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containing 0, 100, 750, and 1,500 ng/ml cotinine. The results were compared with controls obtained for unadulterated spiked normal human urine. The substance or variable was considered not to interfere if there was no change in the NicAlert reading as compared with the control materials. Using these criteria, none of the substances or variables were found to have a positive or negative effect on the NicAlert readings."

The study design and methodology reflect an inaccurate characterization of dipstick performance. The study’s data analysis is predicated in part on an assumption (with no basis) that NicAlert dipsticks can accurately detect a difference in cotinine levels as low as 0.4 ng/ml. For example, in the Supplementary Data Appendix C, a NicAlert reading of “1” (indicating a cotinine concentration of 10–30 ng/ml) for a sample with an LC/MS result of 9.649 ng/ml is classified as a false positive. On that basis, the study’s samples and LC/MS readings would all similarly have to be considered erroneous because they all deviate from each other on a percentage basis by greater than that (>3.5%).

The main uses of the dipstick tests are for the detection and semiquantitation of smoking, and TobacAlert is also useful for detecting SHS at levels of ≥6 ng/ml urinary cotinine. NicAlert and TobacAlert detect urinary and saliva cotinine in seven ranges: 0–10 (0–6 for TobacAlert), 10–30 (6–30 for TobacAlert), 30–100, 100–200, 200–500, 500–1,000, and >1,000 ng/ml. The dipsticks are not intended to be as accurate as LC/MS (and certainly cannot reliably detect levels of SHS lower than 6 ng/ml), but the dipsticks have recognized utility as inexpensive and convenient point-of-care tools for assessment of SHS exposure in the above range. There are clearly labeled limitations to the uses of semi quantitative dipsticks for quantitation; however, the NicAlert and TobacAlert products perform very reliably in the ranges as indicated in their labeling.

There have been a large number of arms-length independent confirmatory studies of these dipstick products performed at reputable laboratories around the world (Bernert et al., 2005; Cooke et al., 2008; Gariti et al., 2002; Montalto et al., 2007; Parker et al., 2002). These published studies have none of the methodological deficiencies that confound any interpretation of the results of Best et al. (2010).

References


