Comparison of SemiQuantitative Cotinine Values Obtained by the DRI Immunoassay and Values Obtained by a Liquid Chromatography–Tandem Mass Spectrometry-Based Method: The DRI Immunoassay is Suitable for Screening Purposes Only Because Semiquantitative Values May Be Unreliable

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Background: DRI cotinine assay is suitable only for screening for cotinine in urine specimens. We studied the reliability of DRI cotinine semiquantitative values by comparing them with the cotinine concentration obtained with a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. **Methods:** Semiquantitative cotinine concentrations in 39 urine specimens obtained by the DRI immunoassay were compared with cotinine concentrations obtained by LC-MS/MS. **Results:** The DRI

cotinine assay consistently overestimated cotinine values obtained by the LC/MS/MS method ($y = 1.1529x + 252.24$, $n = 39$, $R^2 = 0.8899$) indicating that semiquantitative values obtained using the DRI assay may be unreliable. However, no false-negative results were observed using the DRI assay. **Conclusion:** DRI cotinine assay is suitable only for screening cotinine in urine specimens. *J. Clin. Lab. Anal.* **30**:1106–1109, 2016. © 2016 Wiley Periodicals, Inc.

Key words: cotinine; DRI Assay; LC/MS/MS; positive Bias

INTRODUCTION

Cotinine is a useful biomarker of tobacco use because most nicotine entering the body is converted into cotinine. Approximately 10–15% cotinine is excreted into the urine while remainder is further transformed into cotinine glucuronide, trans-3-hydroxycotinine, and trans-3-hydroxy cotinine glucuronide. The half-life of cotinine is longer than nicotine making it a preferred marker of tobacco use and exposure. Although cotinine can be measured in various biological specimens, urine is the preferred specimen (1).

Many insurance providers screen urine specimens for possible presence of cotinine to determine whether an individual is a nonsmoker or smoker. In general, the insurance rate may be higher for smokers. More recently, many companies have adopted a nonsmoking policy and prefer to hire nonsmokers. Urinary cotinine measurement is used for screening of new employees to determine their smoking status. Various

immunoassays are available for determination of cotinine concentration in urine. In addition, a urine dipstick analysis (NickAlert) is also available for cotinine screening (2). In general, 500 ng/ml of cotinine concentration is widely adopted in many laboratories to distinguish smokers from nonsmokers. In one study, the authors reported that a cutoff cotinine concentration of 550 ng/ml can differentiate between passive smokers from active smokers (3). Eremin proposed a urinary cotinine cutoff concentration of 500 ng/ml to differentiate nonsmokers from smokers (4).

The DRI cotinine immunoassay is widely used for determination of cotinine in urine specimens, and this

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assay can also be used in semiquantitative mode. We compared semiquantitative cotinine values obtained by this assay with cotinine values determined by LC-MS/MS to check validity of semiquantitative values as determined by the immunoassay.

MATERIALS AND METHODS

The DRI cotinine assay was obtained from Thermo Fischer (Freemont, CA), and assays were run using Olympus 2700 analyzer following the manufacturer's recommendation. The DRI cotinine assay is a homogeneous enzyme immunoassay based on competition between cotinine labeled with glucose 6-phosphate dehydrogenase and free cotinine present in the urine for binding with limited mouse monoclonal cotinine antibodies present in the reaction mixture. The response of the assay is measured spectrophotometrically at 340 nm. For semiquantitative determination of cotinine, the analytical measurement range is 300–2000 ng/ml. The assay is not designated for quantitative measurement of cotinine in urine specimens.

In our laboratory, the DRI cotinine assay is used for screening purposes only and if an accurate cotinine concentration is desired then the concentration of cotinine is measured using a liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based method. We use a cutoff concentration of 500 ng/ml as recommended by the manufacturer which is higher than the lower limit of quantitation (also recommended by the manufacturer) in our laboratory. We do not use this assay for semiquantitative measurement and as a result do not report result as 1 + , 2 + , 3 + etc., but LC/MS/MS is available for quantification if requested by a physician. However, for this investigation, we used DRI cotinine assay in the semiquantitative mode following the manufacturer's recommendation. Total CV of the semiquantitative assay during this investigation was <10%. For specimens with semiquantitative values over 2000 ng/ml, specimen was reanalyzed after 1:1 dilution with normal saline.

For the analysis of cotinine using LC-MS/MS, an aliquot of urine specimen was diluted 10 times with chromatography grade water and internal standard mix. Cotinine-d3 was used as the internal standard for quantitation. Cotinine was quantitatively measured using LC-MS/MS along with qualitative detection of other nicotine metabolites. Detection and quantitation of nicotine and its metabolites including cotinine using LC-MS/MS have been previously described (5–7). The assay developed for quantitation of cotinine in this report is a modification of these methodologies which has been described before (8). Briefly, liquid

chromatography was performed using a thermostatically controlled column compartment with an Agilent Poroshell 120 SB-C18 reverse phase column (2.1 × 100 mm 2.7 micron, P.N. 685775-902A; Agilent Technologies, Palo Alto, CA). A 6400 series Agilent triple quadrupole mass spectrometer was used for ion monitoring and quantitation. The liquid chromatography was operated at a flow rate of 0.5 milliliters per minute. The starting gradient conditions were 15% mobile phase B, with a ramp to 50% B at 0.5 min followed by a ramp to 95% B at 1.25 min. The gradient returned to 15% mobile phase B at 1.5 min followed by a 1 min equilibration time. The column compartment was heated to 50°C. The mass spectrometer was operated in positive ion mode with multiple reaction monitoring (MRM) mode. The precursor ion for determination of cotinine was m/z 177.1, and product ions were m/z 98.0, 80.1, and 53.2. The precursor ion for the internal standard was m/z 180.1, and the product ion was m/z 80. The analytical measurement range of the assay is 20 to 2000 ng/ml. Usually, total CV for this assay is 7.5% or lower. For values over 2000 ng/ml, specimens were reanalyzed after 1:1 dilution.

RESULTS

We analyzed 39 urine specimens containing various amounts of cotinine that were submitted to our laboratory for cotinine analysis. Plotting cotinine values obtained by the LC-MS/MS method on the x -axis and semiquantitative values obtained by the DRI immunoassay axis on the y -axis, the following regression equation was observed:

$$y = 1.1529x + 252.24 (n = 39, r = 0.8899)$$

The regression equation indicated that there was significant positive bias (both proportional and constant bias) when cotinine in urine specimens was measured by the DRI immunoassay (Fig. 1). Therefore, semiquantitative values obtained by the DRI cotinine assay may be unreliable. However, no false-positive result was observed using the DRI assay indicating that this assay is suitable for screening. Interestingly, although analytical measurement range of the DRI assay is 300–2000 ng/ml, the DRI assay produces semiquantitative values at concentrations lower than 300 ng/ml. When the semiquantitative values produced by the DRI assay at a concentration 102 ng/ml but well below 300 ng/ml, the true presence of cotinine in the specimen can be verified by the LC-MS/MS assay (Table 1). However, for semiquantitative values below 100 ng/ml, the presence of cotinine in the urine specimen cannot be

Fig 1. Regression equation obtained by plotting cotinine concentrations obtained by the LC-MS/MS method in the x-axis, and semiquantitative cotinine values obtained using the DRI cotinine immunoassay

TABLE 1. Urine specimens where cotinine values obtained by the DRI assay were between 102 and 125 ng/ml, and the presence of cotinine was confirmed by LC-MS/MS

Specimen #	Cotinine ng/ml	
	DRI	LC-MS/MS
1	122	80.8
2	100	42.4
3	122	68.9
5	125	65.8
6	114	69.1
7	116	85.2
8	118	40.7
9	102	40.1

confirmed in approximately 50% of specimens using the LC/MS/MS. The range 102–125 ng/ml was chosen arbitrarily for the table to demonstrate that when semiquantitative value by the DRI assay was between 102 ng/ml and 125 ng/ml, the true presence of cotinine in the urine can be confirmed using LC/MS/MS.

DISCUSSION

The most commonly used biomarker of exposure to tobacco is cotinine, and its measurement in urine is recommended for assessment of active tobacco smoking, monitoring environmental exposure of tobacco smoke as well as evaluating individuals undergoing smoking cessation program. Although 500 ng/ml is widely used as the urinary cotinine cutoff

concentration, several publications indicate that a lower cutoff may be more appropriate. Man et al. using gas chromatography–mass spectrometry suggested that an optimum cutoff urine cotinine level of 25 ng/ml could differentiate a nonsmoker from a smoker (9). In another study, the authors reported that at the optimal urinary cotinine cutoff concentration of 82 ng/ml, the sensitivity was 95.2%, and specificity was 96.6% to differentiate nonsmoking pregnant women from smokers (10). Kim et al. used a cutoff concentration of 50 ng/ml of urinary cotinine in their study because that cutoff level is most appropriate for the applications of those authors (11). Therefore, detecting cotinine at a lower cutoff level using immunoassays may be very helpful.

Our study indicates that the DRI cotinine immunoassay is useful for screening purposes only because the semiquantitative values did not compare well with the cotinine concentration obtained by the LC-MS/MS. Therefore, the semiquantitative values should not be used for interpretation of smoking status. Although our preliminary results indicate that screening cutoff may be lower to 100 ng/ml, more studies are needed to establish that this assay can be used for screening of cotinine at much lower concentration recommended by the manufacturer.

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