WESTERN SLOPE LABORATORY

Is Your Urine Really Dilute? An Analysis of Normal Urine Creatinine Measured by HPLC-UV Below the Cutoff Limit of 20 mg/dL

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Introduction

In clinical and forensic toxicology laboratories it is important to identify random urine samples submitted for drug testing for adulteration by using creatinine and specific gravity analysis. In normal urine, the reference range for creatinine is 37-300 mg/dL (female); 44-250 mg/dL (male) (1). While for specific gravity, the reference range is 1.002-1.030 (1). However, there are many factors that independently influence the concentration of creatinine in a urine sample. Therefore, it is possible that many samples may be determined to be dilute according to SAMHSA guidelines (2) when, in fact, a creatinine value of less than 20 mg/dL is normal for that donor. A simple method using cation-pairing high pressure liquid chromatography with a ultra-violet detector (HPLC-UV) was used in this study to determine the creatinine concentration in 4,595 advanced toxicology urine samples. This poster will discuss the use of creatinine concentration data from this population of samples and address concerns of the SAMHSA cutoff for creatinine.

Materials and Methods

The method used for this study was cation-pairing HPLC-UV (Waters 2487). Analysis was performed using a Pinnacle DB column (Restek). For detection of creatinine, the wavelength was set at 236 nm and the flow rate was isocratic at 0.7 mL/min. The mobile phase consisted of water-acetonitrile containing 10 mmol/L of the sodium salt of 1-octanesulfonic acid (Sigma-Aldrich) as the cation-pairing agent. The pH of the mobile phase was adjusted to 3.2. Urine samples were prepared by diluting centrifuged (220 x g for 5 minutes) urine with mobile phase (final dilution, 1:100 by volume). This method allows for a large linear range of quantitation in the physiologically relevant range of 0.1-500 mg/dL and limits of detection as low as 0.05 mg/dL. Twenty samples from the less than 20 mg/dL population were also tested for specific gravity using common colorimetric urine adulteration test strips which allowed for values to three decimal places.

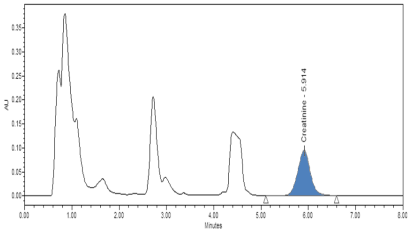

Figure 1: Chromatogram of creatinine in urine using this HPLC-UV method. Concentration of creatinine measured to be 173 mg/dL.

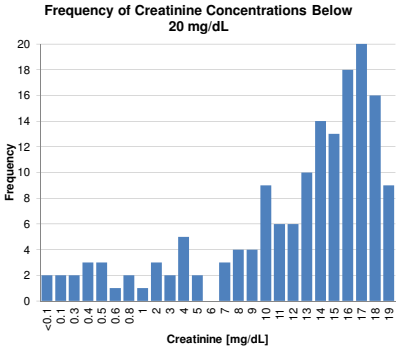

Figure 2: Frequency distribution of the samples with a creatinine concentration of less than 20 mg/dL.

	n	Min	Max	Average	Mode
Total	160	< 0.1	19	12.3	17
Female	112	< 0.1	19	12.5	17
Male	48	< 0.1	18	11.9	16

Table 1: Statistics of 160 advanced toxicology urine samples under the cutoff value [mg/dL].

Results

Figure 1 shows an example of a chromatograph of a sample containing creatinine. In the 4,595 sample population, there were 160 (3.48%) samples which had a creatinine concentration of less than 20 mg/dL. Figure 2 shows the frequency distribution of these 160 samples. Additional descriptive statistics of the population of samples under the SAMHSA cutoff value can be found in Table 1. Also, 20 samples under the SAMHSA cutoff were tested for specific gravity. This data allowed us to look closer at the specific definitions according to SAMHSA of dilute or substituted urine (2), as shown in Figure 3. There were 8 sets of donors with multiple samples under the SAMHSA cutoff value, consisting of 17 samples. These 17 samples had a creatinine ranging from 0.5 mg/dL to 19 mg/dL and an average of 11 mg/dL. Full data from these donors can be found in Figure 4.

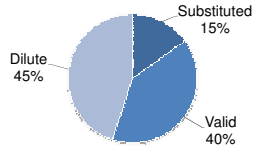

Figure 3: Distribution of samples tested for specific gravity (n=20).

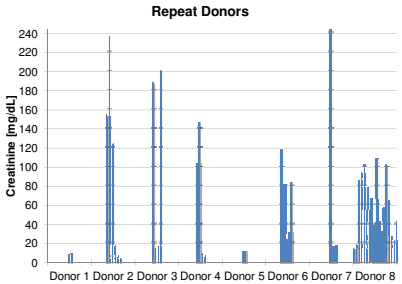

Figure 4: Distribution of urine-creatinine samples from donors with multiple samples under the cutoff value

Discussion

There are many different factors that can influence the creatinine concentration in urine. These factors include but are not limited to: gender, muscle mass, diet, fluid consumption, and several clinical conditions (3). Therefore, we believe that because of these or a combination of these factors, a creatinine concentration of less than 20 mg/dL is not always caused by *in vitro* dilution. The high percentage of females under the SAMHSA cutoff limit shows how a combination of these factors can influence the creatinine in urine. When considering the samples tested for specific gravity in addition to creatinine, 8 (40%) of the samples would be considered valid samples. This illustrates the fact that specific gravity is also important for identifying sample adulteration. Finally, the data from the repeat donors is a good example of the wide range of values one person can have. Figure 4 is able to show how creatinine can have a different range from person to person.

Conclusions

Due to the many factors that can influence the creatinine concentration it is possible that samples under the SAMHSA cutoff of 20 mg/dL do not always implicate sample adulteration.

References

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