The Defined HHS/DOT Substituted Urine Criteria Validated Through a Controlled Hydration Study

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Abstract

This controlled hydration study was designed to validate the substitution criteria used by the Substance Abuse and Mental Health Services Administration (SAMHSA) to classify a workplace urine specimen as inconsistent with normal human urine. Study participants (n = 56) ingested at least 80 oz (2370 mL) of fluid over a 6-h period, 40 oz during the first 3 h (DOT hydration protocol) and 40 oz during the second 3-h period. Urine specimens (n = 498) were collected upon awakening, just prior to hydration, at the end of each hour of the 6-h test period, and upon awakening the next day. No urine specimen satisfied the paired substitution criteria of urine creatinine ≤ 5.0 mg/dL and specific gravity ≤ 1.001 or ≥ 1.020. Seventy-three percent of the participants produced at least one specimen meeting the criteria for dilute urine: urine creatinine < 20.0 mg/dL and specific gravity < 1.003. Fifty-five percent of the participants produced at least one dilute urine specimen during the first 3 h of hydration. In conclusion, this controlled hydration study supports the criteria set by SAMHSA for classifying a specimen as substituted (inconsistent with normal human urine).

Introduction

In February 2000, the U.S. Department of Health and Human Services (HHS), SAMHSA, published the National Laboratory Certification Program (NLCP) State of the Science—Update #1, titled Urine Specimen Validity Testing: Evaluation of the Scientific Data Used to Define a Urine Specimen as Substituted (1). In this document, criteria were selected to classify a workplace drug testing specimen as substituted, that is, not consistent with normal human urine. Based on a published review of four types of studies—normal random urine reference ranges, published clinical studies involving the analysis of random urines, medical conditions resulting in overhydration, and water loading studies—cut-offs for urine creatinine and specific gravity were selected (2). A substituted specimen was defined as a urine creatinine ≤ 5.0 mg/dL and a urine specific gravity ≤ 1.001 or ≥ 1.020.

To validate the defined HHS/Department of Transportation (DOT) substituted specimen criteria, a controlled hydration study was undertaken. Because very few of the published studies used to derive the substituted specimen criteria had paired urine creatinine/specific gravity data, this study produced paired data on each study participant. Because of the concern over small muscle mass and gender and their relationship with low urine creatinine production, the majority of the participants were female and several had a low body mass index (BMI). One limitation of urine specific gravity involves the kidneys’ ability to produce urine of specific gravity ≤ 1.001 during states of excessive hydration. This study required participants to ingest a large quantity of fluid over a short time period to determine the dilutional characteristics of urine produced according to the DOT hydration protocol. Per this hydration protocol, those persons unable to void at least 45 mL of urine upon initial request for a workplace drug screen are allowed to ingest a maximum of 40 oz of liquid during a 3-h period (3).

Materials and Methods

Study participants

Study participants [n = 54, 41 females and 13 males, aged 19–56 years (mean age 32 years), 33 whites, 13 Hispanics, 5 blacks, and 3 Asians, 3 pregnant, BMI (mean 24.49 kg/m², S.D. 4.43 kg/m², range 17.58–36.02 kg/m²) (healthy reference interval 18.5–24.9 kg/m²)] were enrolled in a controlled hydration study. Two participants (2 white females) repeated the study protocol on another day to bring the total number of participants to 56. All participants gave informed consent concerning the hydration protocol. Each participant was instructed to adhere to the protocol and to perform the following tasks: document fluid intake volume, time of fluid consumption, type of fluid consumed, and collect and measure all voided urine volume as instructed. Demographic information requested from each participant included age, gender, weight, height, race, and pregnancy status. Additionally, participants...
were asked to provide a list of their prescribed medications as well as to document any other medication taken in the 24 hours prior to beginning the study. Also, participants provided information on their dietary habits, especially if they were vegetarian because vegetarians have physiologically decreased serum and urine creatinine values. Each participant was given a detailed instruction sheet, a documentation form to record all the information required for the study, and supplies (including collection cups, test bottles, and labels).

Hydration protocol

Each participant was asked to document the volume and nature of anything ingested after midnight of the first study day. Additionally, the volume of all urine voided during the first night was recorded. Upon awakening, the first morning void was collected. Thereafter, each participant was instructed to follow their normal morning routine, including drinking whatever they wanted in their usual amounts, as long as fluid intake and output was faithfully recorded. At a time of their choosing, participants began the hydration protocol. Each participant was asked to ingest at least 80 oz (2370 mL) of fluids of their choosing over a 6-h period, with 40 oz of liquid consumed during the first 3 h and at least 40 oz consumed during the second 3-h period. Specifically, for the first 3 h of the protocol, each participant was requested to drink 13.3 oz (395 mL) of fluid at the beginning of each hour. For the second 3-h period, each participant was asked to ingest at least 13.3 oz (395 mL) at the beginning of each hour, except now participants were encouraged to drink more than the minimally required 13.3 oz of fluid per hour. Each participant collected urine specimens upon awakening on the day of the hydration protocol, just prior to the start of the hydration protocol, at the end of each hour of the 6-h hydration protocol test period, and upon awakening on the morning following the test day. This last specimen was collected to determine if the creatinine concentration and specific gravity had returned to levels consistent with pre-test values. A portion of each void was poured into a bottle labeled with the participant’s unique identification number as well as a number to designate the sequence of the urine void. The participant sealed each bottle. Any voids other than at the end of each hour of the hydration protocol were measured for volume but not tested. Any scheduled void that was missed was documented. Per the protocol, each participant was asked to submit 9 urine specimens for an expected total of 504 specimens. However, four participants did not submit all specimens requested: three subjects forgot to collect the first morning void, and the fourth subject did not provide three specimens because he/she could not complete the second 3-h protocol, complaining that it was too much fluid to drink. In all, 498 specimens were collected. Each specimen was divided into two aliquots for subsequent testing.

Methods

The urine aliquots were analyzed within 1–4 days of collection (Kroll Laboratory Specialists, Gretna, LA) for urine creatinine by an automated modified Jaffé method on the Hitachi 747 (Roche Diagnostic Systems, Indianapolis, IN) and for urine specific gravity on the Atago model UG-1 (Vee Gee Scientific, Inc., Kirkland, WA) electronic refractometer. Both instruments were calibrated with known standards and the calibration checked with quality control materials. The performance of the creatinine assay at low concentrations was assessed by the inclusion of a 3.2-mg/dL creatinine quality control material (mean 3.222 mg/dL, S.D. 0.391 mg/dL, C.V. 12.13%). The performance of the refractometer at the low end was assessed by testing deionized water at 1.000 (mean 1.000, S.D. 0.0, C.V. 0%). Statistics were calculated using Microsoft Excel software (Microsoft Corp., Roselle, IL).

Results

The average volume of fluid ingested during the hydration protocol was 3104 mL (S.D. 980 mL, range 1004–5320 mL). Twelve participants (5 men and 7 women) consumed over 1 gal (3.79 L) of fluid by the end of their test periods. Two participants were unable to consume the minimum amount of fluid originally intended (80 oz or 2370 mL spread evenly over the 6 h). The remainder of the participants consumed at least the minimum requested per the protocol. Though both the creatinine and specific gravity data indicated a slight tendency towards decreasing values with increasing volume ingested, no significant correlation was evident. The lowest creatinine/specific gravity data pair for each subject versus their
total ingested fluid volume is presented in Figures 1 and 2.

A statistical summary of the participant urine data by collection time is presented in Table I. Shown in Figures 3 and 4 are plots of the mean creatinine and specific gravity results, respectively, at each of the nine collection time points. These plots depict the trend to lower values of creatinine and specific gravity with hydration and the return to baseline values within 24 h.

During the first 3 h of the hydration study in which the study participants followed the DOT hydration protocol, the lowest urine creatinine result was 5.2 mg/dL (data pair: 5.2 mg/dL creatinine and 1.001 specific gravity), achieved at the end of the third hour of hydration. The lowest specific gravity seen during the first half of the hydration study was 1.001 (13 data pairs: 1.001 specific gravity and creatinines ranging from 5.2 to 15.4 mg/dL), also achieved at the end of the third hour of hydration.

For the second half of the hydration study where participants were encouraged to drink more than 40 oz of fluid over three hours, the lowest urine creatinine result of 5.1 mg/dL (one data pair: 5.1 mg/dL creatinine and 1.001 specific gravity) occurred at the end of the fifth hour of hydration. The lowest specific gravity was 1.000 (5 data pairs: 1.000 specific gravity and creatinines ranging from 5.1 to 7.8 mg/dL), also achieved at the end of the fifth hour of hydration.

### Table I. Data Pairings for the Lowest Hydration Results

<table>
<thead>
<tr>
<th>Creatinine Concentration (mg/dL)</th>
<th>Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1*</td>
<td>1.001</td>
</tr>
<tr>
<td>7.8</td>
<td>1.000*</td>
</tr>
</tbody>
</table>

* The lowest result of the pair.

For the second half of the hydration study where participants were encouraged to drink more than 40 oz of fluid over three hours, the lowest urine creatinine result of 5.1 mg/dL (one data pair: 5.1 mg/dL creatinine and 1.001 specific gravity) occurred at the end of the fifth hour of hydration. The lowest specific gravity was 1.000 (5 data pairs: 1.000 specific gravity and creatinines ranging from 5.1 to 7.8 mg/dL), also achieved at the end of the fifth hour of hydration.

![Figure 1](image1.png)  
**Figure 1.** The creatinine from the lowest achieved creatinine/specific gravity pair for each subject is plotted versus his/her total fluid volume.

![Figure 2](image2.png)  
**Figure 2.** The specific gravity from the lowest achieved creatinine/specific gravity pair for each subject is plotted versus his/her total fluid volume.

![Figure 3](image3.png)  
**Figure 3.** Plot of the mean creatinine concentrations at each of the nine collection time points. Specimen 1 was the first morning void, specimen 2 was the baseline specimen collected just prior to the beginning of the hydration protocol, specimens 3–8 were collected at the end of each hour of the 6-h hydration protocol, and specimen 9 was the first morning void collected the morning after.

![Figure 4](image4.png)  
**Figure 4.** Plot of the mean specific gravity results at each of the nine collection time points. Specimen 1 was the first morning void, specimen 2 was the baseline specimen collected just prior to the beginning of the hydration protocol, specimens 3–8 were collected at the end of each hour of the 6-h hydration protocol, and specimen 9 was the first morning void collected the morning after.

![Figure 5](image5.png)  
**Figure 5.** Graphical representation of those specimens (n = 113) produced during the hydration protocol that met the criteria for a dilute specimen, defined as a urine specimen with creatinine concentration < 20 mg/dL and specific gravity < 1.003.
gravity during the second portion of the hydration protocol was 1.000 (one data pair; specific gravity 1.000 and 7.8 mg/dL creatinine), which also occurred at the end of the fifth hour. The lowest data pairings for each analyte found in this study are presented in Table II. The delay in the production of the most dilute urine specimens under this hydration protocol supports previous findings where the relationship between urine creatinine and fluid ingestion is neither linear nor immediate. With a large intake of fluid, there is a 2:1 disparity between the intake and the void volumes because the kidneys are not capable of processing large quantities of fluid within a short time after ingestion. With normal fluid intake, the void volume approximates ingestion. After fluid ingestion, 2–5 h are typically required to see a significant change in urine creatinine (2).

Out of 498 total urine specimens, 170 specimens (34.1%) had urine creatinine concentrations < 20 mg/dL (48/56, 86% of participants), 115 specimens (23.1%) had specific gravities < 1.003 (41/56, 73% of participants), and 113 specimens (22.7%) met the criteria for a dilute specimen under these extreme hydration conditions with both a creatinine result < 20 mg/dL and a specific gravity result < 1.003 (42/56, 75% of participants). A graphical representation of the specimens classified as dilute is presented in Figure 5. None of the 498 specimens met the paired criteria for substitution, a creatinine ≤ 5.0 mg/dL and a specific gravity ≤ 1.001 or ≥ 1.020. A summary of the specimens meeting dilute criteria by gender and race is given in Table III.

Though both creatinine and specific gravity are used as criteria to define a dilute specimen, they are physiologically different. Creatinine is a metabolic waste product whose production and excretion into urine are relatively constant from day to day. Urine specific gravity is the weight of urine compared with the weight of an equal volume of distilled water at the same temperature. The specific gravity of urine varies greatly with fluid intake and hydration status. Despite these differences, linear regression statistics of the study data showed good correlation, yielding a linear regression equation of creatinine versus specific gravity ($y = 0.0001x + 1.003$) and a correlation coefficient of 0.91 (Figure 6).

Results from the water-loaded participant with the lowest BMI, 17.58 kg/m², were graphed to depict the relative trending of creatinine and specific gravity with water ingestion (Figure 7). Though the amount of total fluid ingested increased slightly with BMI, there was not a

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**Table III. Specimens Produced during the Hydration Protocol that are Classified as Dilute and Categorized by Gender and Race**

<table>
<thead>
<tr>
<th>Participants (gender and race)</th>
<th>Number</th>
<th>Participants producing one or more dilute specimens</th>
<th>Total dilute specimens by race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Male subtotals</td>
<td>13</td>
<td>7 (53.8%)</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>27</td>
<td>23</td>
<td>70</td>
</tr>
<tr>
<td>Black</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female subtotals</td>
<td>43</td>
<td>35 (81.3%)</td>
<td>103</td>
</tr>
<tr>
<td>Participant totals</td>
<td>56</td>
<td>42 (75%)</td>
<td>113</td>
</tr>
</tbody>
</table>
significant correlation ($r^2 = 0.145$). No correlation was evident between the lowest achieved creatinine/specific gravity pair and the subject’s BMI (BMI/creatinine $r^2 = 0.005$; BMI/specific gravity $r^2 = 0.003$).

Conclusions

In comparison with other published hydration studies that collected urine creatinine and/or specific gravity data, this protocol was more extensive, enrolling 56 participants, compared with the published studies whose subject enrollment varied from 1 to 23 (2). Additionally, the present study recruited a diverse subject population (2).

Per this hydration protocol, participants were requested to ingest at least 2.37 L of fluid over 6 h or 0.4 L/h. This fluid loading is comparable to published hydration studies where the fluid doses ranged from 0.2 to 4.0 L/h for a total fluid load ranging from 1.0 to 7.4 L/h (2). Though the total amount ingested by this study’s participants (1.0–5.3 L) is comparable to the previously mentioned published studies, the dosing level is on the low end because one purpose of this study was to assess the dilution characteristics of urine produced under the DOT hydration protocol.

In this controlled hydration study, none of the 498 urine specimens collected from the 56 study participants met the paired criteria that define a specimen as substituted by HHS/DOT: creatinine $\leq 5.0$ mg/dL and specific gravity $\leq 1.001$ or $\geq 1.020$. Interestingly, 41 (73%) of the study participants were able to produce at least 1 specimen during the hydration protocol that met the HHS/DOT criteria for dilute urine: creatinine $< 20.0$ mg/dL and specific gravity $< 1.003$. The study supports the reasoning that excessive hydration produces urines that meet the HHS/DOT dilute urine criteria.

Even those participants of small muscle mass as indicated by their BMI values did not produce urine specimens during the hydration protocol that met the substituted criteria. In fact, of those participants having BMI values $\leq 20$, only 56% could produce urine specimens meeting the dilute criteria during hydration. Of the three participants with a BMI $< 18.5$, only one produced just one specimen that met the dilute criteria.

Specific gravities $\leq 1.001$ can be achieved with hydration. The specific gravities of 46 of the 498 submitted specimens (9.2%) collected during the hydration protocol were $\leq 1.001$. In this study, very dilute urine specimens were produced. However, no participant provided a specimen that met the HHS/DOT substituted urine criteria.

This controlled hydration study supports the contention that the DOT hydration protocol does not cause a donor to produce a specimen that meets HHS/DOT substituted specimen criteria. Specimens meeting the criteria for a dilute specimen during the first 3 h of hydration were produced by 31 (55%) of the study participants.

Most importantly, this study supports the substitution criteria used by HHS/DOT; none of the specimens collected during both phases of this study had a creatinine concentration $\leq 5.0$ mg/dL and specific gravity $\leq 1.001$.

Acknowledgments

 Portions of this paper were originally presented as a poster at the 2000 Society of Forensic Toxicologists, Inc. meeting on October 4, 2000, in Milwaukee, WI. An abstract of this study, in conjunction with the 2000 SOFT meeting, was published in the Journal of Analytical Toxicology [25: 367 (2001)].

References