TECHNICAL NOTE

Joseph J. Saady,[1] Ph.D.; Alphonse Poklis,[1] Ph.D.; and Harry P. Dalton,[1] Ph.D.

Production of Urinary Ethanol After Sample Collection

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ABSTRACT: As the interest in urine drug testing grows, ethanol is frequently included in drug-abuse screening. Collection of urine for drug testing is less invasive than blood collection and is used to screen employees in a large cross-section of occupations. Because alcohol can be produced from carbohydrates via fermentation, our interest was to determine: (1) if ethanol could be produced in glucose-positive urine (2) under what microbiological conditions would this process occur, and (3) would the urine ethanol concentration be significant.

Fourteen urine specimens were selected from the Urinalysis Laboratory of a large medical center. All specimens were tested for ethanol concentration on the day of voiding and were found to be negative (<0.01 mg/100 mL). Urine glucose concentrations ranged from 0 to ≥ 2000 mg/dL. Microbiological examinations were performed on all specimens.

Storing the samples at room temperature, five of the specimens produced ethanol over the time course of the study (1 to 21 days) in concentrations ranging from 0.036 to 2.327 g/100 mL. Yeast was identified in the five glucose positive urine samples producing ethanol. Six glucose positive urine samples that did not produce ethanol were found to be yeast negative.

Findings indicate that significant ethanol concentrations can develop from glucose and yeast positive urine, after the day of voiding.

KEYWORDS: toxicology, ethanol, urine, fermentation, yeast

Fermentation of carbohydrates by yeast is the oldest synthetic chemical process known, and is used for the production of alcohols, particularly ethyl alcohol. Indeed, ethyl alcohol is the oldest synthetic organic chemical and, along with carbon dioxide, is the product of the fermentation process (that is, the enzymatically controlled anaerobic breakdown of carbohydrates) [*1–4*]

In this age of urine drug testing, forensic toxicologists need to be reminded of the fermentation process, particularly when a urine ethanol result may adversely reflect in-

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[1]Department of Pathology, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA.

formation concerning an employee in a regulated occupation (for example, transportation). The collection and testing of urine for drugs of abuse is currently accepted, and urine collection is less invasive than blood collection. Screening urine for ethanol has been considered an acceptable (albeit controversial) testing strategy for the detection of ethanol. Some have even proposed the use of urinary concentrations as a predictor of blood and breath ethanol [5].

Our intention in this study was to observe urine ethanol concentrations over time, and to determination how urinary glucose, if present, affected the ethanol concentration. We also cultured the urine specimens in order to document the microbiological flora present, and compared microbiological identification to ethanol production.

Materials and Methods

Urine specimens were obtained from the Urinalysis Laboratory, which receives specimens from throughout a large University Medical Center (Medical College of Virginia) from various medical services (for example, emergency rooms, general medical clinics, and etc.). Urine specimens were selected in a random fashion so as to include a range of glucose concentrations from negative through ≥2000 mg/dL. All specimens were tested for glucose and ethanol on the day of voiding (day 0). Ethanol concentrations were subsequently determined on days 2, 14, and ≥20. Microbiological identification was begun approximately at Day 7. Urine samples were selected at random from the specimens in the laboratory, which were submitted form several services. No information is available regarding the clinical or metabolic state of the individual patient. Conceivably, some were hospital patients, some were routine clinic patients, and some were emergent for various reasons. All urine samples were stored at room temperature from the time of collection throughout the duration of the study. No preservative (for example, fluoride) was used.

Analytical Methods

Urinary glucose determinations were performed with the Ames Clinitek Auto 2000, which is an automated urine chemistry analyzer. Urinary glucose concentrations are read by the instrument in mg/dL and semi-quantitative results are printed as one of the following values: negative, 100, 250, 500, 1000, or ≥2000. The instrument uses a halogen lamp and analyzes at defined wavelengths, the color and intensity of light reflected from a reacted reagent area. The intensity of the reflected light is semi-quantitatively proportionate to the amount of glucose in the urine specimen [6].

All ethanol concentrations were performed by gas chromatography using a flame ionization detector and a 2 mm × 0.9 m glass column. The packing material was 5% Carbowax 20M on 60/80 mesh Carbopack B (Supelco) and column temperature was 75°C. Both the ethanol and the 1-propanol internal standard eluted from the column within five minutes. Also, if methanol, acetone, and/or isopropanol were present, each would be qualitatively and quantitatively identified by the gas chromatographic retention time and peak height, respectively.

Microbiological techniques: all urine specimens were planted on blood agar plates and Eosen Methylene Blue agar using the conventional colony count method. A direct gram stain was performed on all urine specimens and these results were correlated with the colony count isolation results. All organisms were identified as outlined by the *Manual of Clinical Microbiology* [7].

TABLE 1—*Ethanol concentrations*[a] *in urine specimens testing positive.*[b]

Sample #	Glucose[c]	Number of Days After Voiding				Microbiological examination[d]
		0	2	14	20+	
J	250	negative	negative	0.079	0.072	Escherichia, yeast[e]
K	1000	negative	0.081	0.388	0.326	Candida albicans
L	≥2000	negative	0.036	—	—	Candida albicans
M	≥2000	negative	0.283	2.327	2.383	Escherichia, Enterococcus, Candida tropicalis
N	≥2000	negative	negative	0.193	0.218	Klebsiella oxytoca, Enterococcus, yeast[e]

[a]Units in g/100 mL.
[b]Cutoff 0.01 g/100 mL.
[c]Units in mg/dL.
[d]Performed by culturing urine.
[e]Determined by gram stain.

TABLE 2—*Specimens testing negative[a] for ethanol[b].*

Sample #	Glucose[c]	Microbiological examination[d]
A	negative	Escherichia, Enterococcus
B	negative	Escherichia
C	negative	Staphylococcus coag negative
D	100	no growth
E	100	Enterococcus, Proteus mirabilis
F	100	Escherichia
G	250	Enterococcus, Staphylococcus aprophyticus
H	250	no growth
I	≥2000	no growth

[a]Cutoff 0.01 g/100 mL.
[b]Testing performed on Days 0, 2, 14, 20+.
[c]Units in mg/dL.
[d]Performed by culturing urine.

Results and Discussion

Fourteen urine samples were tested for glucose, ethanol, and cultured for microbiological flora. The urine glucose concentrations ranged from negative to ≥2000 mg/dL. Ethanol concentrations were measured on day 0 (that is, the day of voiding), and Days 2, 14, and >20. Ethanol concentrations ranged from negative to 2.327 g/100 mL over the total time period. All urinary ethanol concentrations were negative (cutoff 0.01 g/100 mL) on day 0. Nine of the urine specimens remained negative throughout the study. Five of the specimens "produced" ethanol over the time period at concentrations from 0.036 to 2.327 g/100 mL. All urine samples in which ethanol was found also contained glucose ranging in concentration from 250 to ≥2000 mg/dL. All urine samples testing positive for ethanol (>0.01 g/100 mL) also tested positive for the microbiological identification of yeast. A tabular representation of samples found positive and negative for ethanol can be seen in Tables 1 and 2, respectively.

It is significant that ethanol was found in the five urine specimens which contained varying concentrations of glucose and that yeast was identified in those samples. It is not unusual for the Microbiology Laboratory to isolate yeast from urine specimens, as 780 yeast positive urine samples were isolated from 20 000 specimens received during the past year (3.9%). Six urine samples testing negative for ethanol over the time course had no yeast present. Microbiological isolations in these samples resulted in no growth in three of these, and other bacteria present in the remaining three. Bacteria identified belonged to the genera Enterococcus, Proteus, Escherichia, and Staphylococcus.

Toxicologists should be vigilant and cautious regarding urine ethanol testing. Conversion of urinary glucose to ethanol, after sample collection, in the presence of yeast will occur. However, this process will not occur immediately and requires greater than twelve hours, as noted in the current study. Measurable amounts of ethanol were not detected when tested on the day of the voiding, even when the conditions for fermentation existed.

References

[1] Morrison, R. T. and Boyd, R. N., *Organic Chemistry*, 2nd ed, Allyn and Bacon, Inc., Boston, 1967.

[2] Alexander, W. D., Wills, P. D., and Eldred, N., "Urinary Ethanol and Diabetes Mellius," *Diabetic Medicine*, Vol. 5, 1988, pp. 463–464.

[3] Ball, W. and Lichtenwalner, M., "Ethanol Production in Infected Urine," *New England Journal Medicine*, Vol. 301, No. 11, Sept. 1979, p. 614.
[4] Panchal, C. and Tavares, F., "Yeast Strain Selection for Fuel Ethanol Production," *Yeast Strain Selection*, C. Panchal, Ed., Marcel Decker, Inc., New York, 1990.
[5] Biasotti, A. A. and Valentine, T. E., "Blood Alcohol Concentration Determined from Urine Samples as a Practical Equivalent or Alternative to Blood and Breath Alcohol Tests," *Journal of Forensic Sciences*, Vol. 30, No. 1, Jan. 1985, pp. 194–207.
[6] Baker, J., Edlund D., Safdy, M., and Tyhach, R., "Comparison of the Clinical Performance of Ames Clinteck 200 and Clintek 10 Urine Chemistry Analyzers Obtained with Multistix 10 SG Reagent Strips," *Clinical Chemistry*, Vol. 32, No. 6, 1986, p. 1192.
[7] Lennette, E. H., *Manual of Clinical Microbiology*, 4th edition, American Society for Microbiology, Washington, D.C., 1985.

Address requests for reprints or additional information to
Joseph J. Saady, Ph.D.
Dept. Of Pathology
Virginia Commonwealth University
Medical College of Virginia
Richmond, VA 23298-0597