



Sensitivity and specificity of a commercial urinary ethyl glucuronide (EtG) test in heavy drinkers



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ABSTRACT

Introduction: To advance the use of alcohol metabolites as biomarkers in the context of alcohol research, the present study tested the sensitivity and specificity of a commercially available urinary ethyl glucuronide (uEtG) test (DrugConfirm Advanced 80hr EtG) in a clinical research context.

Methods: A community sample of heavy drinkers (N = 68) completed the 30-day Timeline Follow-Back (TLFB) interview and provided a urine sample for uEtG analysis. Analyses of sensitivity and specificity of the uEtG assay were conducted using the following outcomes: (a) past day drinking, (b) past day binge drinking (defined as ≥ 4 drinks for women and ≥ 5 drinks for men), (c) past 3-day drinking, and (d) past 3-day binge drinking.

Results: The majority of participants reported past-3-day drinking (80.9%) and a sizeable minority reported past day drinking (33.8%). While uEtG-based detection of past day drinking and binge drinking was acceptable (sensitivity = 73.91%, and 83.33%; specificity = 80.00% and 66.13%, respectively), detection of any drinking and binge drinking in the past 3 days was poor (sensitivity and specificity of 43.64% and 84.62%, and 39.39% and 62.86%, respectively).

Conclusions: This study contributes to the mixed findings on the validity of EtG tests, which suggest that commercial uEtG tests with conservative detection thresholds are not a reliable alcohol biomarker without corroborating self-report data. Lower detection thresholds are recommended when using uEtG as an alcohol biomarker. Efforts to reach acceptable levels of sensitivity and specificity with commercial assays hold potential to advance the measurement of alcohol intake, overcoming the pitfalls of self-report data.

1. Introduction

Research with alcohol using populations commonly relies on standardized self-report measures to assess an individual's alcohol intake. Recently, interest in biochemical markers of alcohol use has increased, as biomarkers offer advantages over self-report measures to indicate alcohol use independent of recall bias or other motivating factors. However, alcohol biomarkers can be less sensitive than well-standardized self-report measures, and to date, no biomarker has gained widespread acceptance as a primary outcome measure of alcohol use (Kalapatapu & Chambers, 2009; Litten, Bradley, & Moss, 2010; Roberts & McKee, 2018; Shorter et al., 2019). The potential utility of biomarkers for alcohol research are multiple and include serving as an objective outcome measure in treatment settings and research studies, screening for alcohol use in individuals who are unable to provide valid self-report data, and establishing abstinence among individuals

mandated to abstain from drinking (Jastrzebska et al., 2016). However, in order for the promise of alcohol use biomarkers to be realized, practical assays (i.e. those which can be conducted solely within a treatment and/or research setting) with strong sensitivity, specificity, and a clear understanding of their window of detection are needed.

The field of alcohol biomarkers has traditionally relied on indirect markers, such as gamma-glutamyl-transpeptidase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), mean corpuscular volume (MCV), and carbohydrate deficient transferrin (CDT). However, these markers are influenced by a host of inter-individual differences, non-alcohol related illnesses, and tend to capture only long term alcohol use (Wurst et al., 2015). More recently, direct ethanol metabolites have received increased attention as they represent minor pathways of ethanol elimination with the potential for higher sensitivity and specificity than indirect makers. The most frequently used measures include ethyl glucuronide (EtG), ethyl sulphate

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(EtS), and phosphatidylethanol (PEth).

EtG is a phase 2 metabolite of alcohol that can be detected in urine, whole blood, serum, and hair (Foti & Fisher, 2005; Kissack, Bishop, & Roper, 2008; Wurst et al., 2015). EtG can be detected in urine for up to 80 h after ethanol consumption depending on the amount of alcohol consumed (Kissack et al., 2008; Wurst et al., 2015). Few studies have assessed the ability of commercially available urinary EtG tests detect recent alcohol use. One study used a commercial test to detect alcohol use among non-drinking, former drinking, and current drinking HIV-Hepatitis B virus co-infected individuals in Zambia (Vinikoor et al., 2018), while another study used a commercial test to detect light drinking among women of childbearing age (Graham, Beatty, Rosano, Sokol, & Ondersma, 2017). Whereas the first study found support for the use of a point-of-care urinary EtG test using a 500 ng/dl detection cutoff (Vinikoor et al., 2018), the second study, which consisted of a controlled alcohol administration and 3-day follow-up period, found that an uEtG cutoff of > 100 ng/dl (representative of those used by commercial laboratories) was not sensitive enough to reliably detect light-to-moderate drinking beyond a 12-hour window (Graham et al., 2017). Studies that have assessed urinary EtG using more sensitive laboratory measures such as liquid chromatography-mass spectrometry (LC-MS) in alcohol using populations have found that uEtG has good sensitivity and specificity for the detection of recent drinking (Armer, Gunawardana, & Allcock, 2017; Dahl, Voltaire Carlsson, Hillgren, & Helander, 2011; Stewart, Koch, Burgess, Willner, & Reuben, 2013; Wurst, Wiesbeck, Metzger, & Weinmann, 2004). However, uEtG using these advanced laboratory techniques may be too sensitive in that it also captures incidental exposure to other alcohol sources (e.g., hand sanitizer, mouthwash), resulting in false positives (Costantino, DiGregorio, Korn, Spayd, & Rieders, 2006). Additionally, if a urine sample is stored in room temperature for more than 12 h, yeast may convert urine glucose into ethanol, and therefore, EtG (Kissack et al., 2008). Enzyme immunoassays have been developed as an alternative to the costly and potentially overly-sensitive LC-MS method (Böttcher, Beck, & Helander, 2007). This method has high sensitivity and good agreement with LC-MS results. However, the enzyme immunoassay method still requires advanced laboratory instruments which may not be ideal in clinical research contexts.

In sum, there is a strong need for research regarding biomarker assay cutoff values and associated detection windows in research contexts (SAMHSA, 2006, 2012). To advance the use of alcohol metabolites as biomarkers in the context of alcohol research, the present study tested the sensitivity and specificity of a commercially available urine EtG test to detect recent alcohol drinking in a clinical research sample of heavy drinkers.

2. Methods

2.1. Participants

The study protocol and all procedures were approved by the Institutional Review Board of the University of California, Los Angeles. Participants were recruited from the greater Los Angeles community through fliers, online and print advertisements, and social media. Study advertisements targeted individuals who drank alcohol, but did not specify specific study inclusion or exclusion criteria. Inclusion criteria were as follows: (1) heavy drinking, indicated by a score of 8 or higher on the Alcohol-Use Disorders Identification Test (AUDIT) (Saunders, Aasland, Babor, De la Fuente, & Grant, 1993), signifying a hazardous drinking pattern, or drinking 14+ drinks per week for men or 7+ drinks per week for women and as defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA); and (2) between the ages of 21 and 65. Participants (N = 68) for this study were selected as a convenience sample from three completed or ongoing behavioral pharmacology studies of heavy drinkers that collected both uEtG and Timeline Follow-back (TLFB) (Sobell, Sobell, Klajner, Pavan, & Basian,

1986). Study 1 (N = 17) examined the relationship between subjective response and self-administration of alcohol (Bujarski et al., 2018). Study 2 (N = 46) is ongoing and tests the combination of varenicline and naltrexone for smoking cessation among heavy drinking smokers. Study 3 (N = 6) compared treatment-seekers versus non-treatment seekers for alcohol use disorders (AUD) on alcohol cue-reactivity in the laboratory (Venegas & Ray, 2019). Since there was no imposed or implied upper limit on drinking, participants were not motivated to underreport or otherwise misrepresent their alcohol use. Additionally, this data was collected during a behavioral screening visit, and therefore, participants were not informed of necessary inclusion/exclusion criteria regarding their drinking, nor were they influenced by clinician involvement to underreport their alcohol use. Participants were compensated \$30 for completing the behavioral screening visit.

2.2. Procedures

Initial assessment of the eligibility criteria was conducted through a telephone interview. Eligible participants were invited to the laboratory for an in-person assessment. All participants read and signed an informed consent form upon receiving a full explanation of all study procedures. Participants then provided a urine sample for EtG analysis and completed individual differences questionnaires and interviews. All participants were required to test negative for drugs of abuse on a urine drug test (except for marijuana) and to have a BrAC of 0.00 g/dl at the assessment visit. All female participants tested negative for pregnancy.

2.3. Measures

The Timeline Follow-back (TLFB) (Sobell et al., 1986) was used to assess for quantity and frequency of drinking over the past 30 days. The DrugConfirm Advanced 80hr EtG urine drug test, obtained from Confirm Biosciences, was used as a point-of-care test that is commercially available. Urine samples were collected at the beginning of study visit and the uEtG test was analyzed within 5 min of collection. This dipped urine EtG test strip had a detection threshold of 500 ng/ml and provided a qualitative (positive/negative) result, indicated by the number of lines on the test strip (1 = positive, 2 = negative). A threshold of 500 ng/ml is interpreted as indicating previous heavy drinking (1–3 days), light drinking (12–36 h), and excludes most cases of incidental exposure to alcohol (Jatlow et al., 2014; SAMHSA, 2006, 2012). The study team did not disclose the results of uEtG tests to participants, thus reducing possible reporting bias during the TLFB interview.

Measures of alcohol use and alcohol use disorder severity were also collected. The Structured Clinical Interview for DSM-5 (SCID; adapted from (First, Williams, Karg, & RL, 2015)) assessed for lifetime and current AUD. Participants also completed the Alcohol Use Disorders Identification Test (AUDIT (Allen, Litten, Fertig, & Babor, 1997)) and the Penn Alcohol Craving Scale (PACS (Flannery, Volpicelli, & Pettinati, 1999)). Of note, 6 participants in this sample did not complete the AUDIT.

2.4. Data analysis

All analyses were conducted in R version 3.3. Self-reported recent alcohol consumption from the TLFB interview was used as the reference test. Participants were coded based on the four primary variables of interest: past day drinking, past day binge drinking, past 3-day drinking, and past 3-day binge drinking, all taken from the TLFB interview. Binge drinking was defined as ≥ 4 drinks for women and ≥ 5 drinks for men by NIAAA (<https://www.niaaa.nih.gov/alcohol-facts-and-statistics>). Sensitivity and specificity were computed from frequency tables with sensitivity defined as the number of true detected positives/total positives and specificity defined as the number of true detected negatives/total negatives. McNemar's χ^2 tests were also

Table 1
Participant characteristics.

	Mean + SD	Range
Age	36.49 ± 12.62	21–62
Sex (M/F)	41/27	–
Ethnicity	31	–
Caucasian (%)	(45.6%)20	
African American (%)	(29.4%)15	
Latino (%)	(22.1%)11	
Asian (%)	(1.5%)1	
Other/Unknown (%)	(1.5%)	
Total Drinks (30 Days)	97.00 ± 83.73	1.68–375.84
Number of Drinking Days (30 Days)	16.79 ± 9.00	1–30
Drinks Per Drinking Day (30 Days)	5.34 ± 3.55	0.84–21.27
Number of Binge Drinking Days (30 Days)	8.35 ± 8.88	0–30
AUD Diagnosis	17	–
None (%)	(25%)21	
Mild (%)	(30.9%)19	
Moderate (%)	(27.9%)11	
Severe (%)	(16.2%)	
AUDIT ^a	15.03 ± 7.71	1–35
PACS	12.06 ± 6.29	0–29

^a = data is missing from 6 participants.

conducted to determine whether the accuracy of the EtG test reached statistical significance.

3. Results

3.1. Sample characteristics

Of the 68 participants enrolled in this study, 40% (N = 27) were female. The average age was 36.49 (SD = 12.62). On average, participants reported drinking on 16.79 of the past 30 days (SD = 9.00), binge drinking on 8.35 days (SD = 8.88) of the past 30 days, and consuming 5.34 drinks per drinking day (SD = 3.55). See Table 1 for a full description of participant characteristics.

3.2. Sensitivity and specificity of urinary EtG

Analyses of sensitivity and specificity were conducted using the following endpoints derived from the TLFB interview: (a) past day drinking, (b) past day binge drinking (defined as ≥4 drinks for women and ≥5 drinks for men), (c) past 3-day drinking, and (d) past 3-day binge drinking. 33.8% (N = 23) of participants reported past day drinking (average number of drinks consumed in the past day = 1.26 (SD = 2.50)), while 80.9% (N = 55) reported past 3-day drinking (average number of drinks consumed over the past 3 days = 8.43 (SD = 10.57)). Past day binge drinking was reported by 8.8% (N = 6) of participants, while 3-day binge drinking was reported by 48.5% (N = 33) of participants. As shown in Table 2, sensitivity and specificity for the urine EtG test were acceptable for detecting past day drinking (sensitivity = 73.91%, specificity = 80.00%, $\chi^2(1) = 18.73$, $p < 0.001$). Specifically, 9 participants reported no past-day drinking during the TLFB interview but had a positive uEtG result. Similarly, sensitivity and specificity for the uEtG test were acceptable for detecting past day binge drinking (sensitivity = 83.33%, sensitivity 66.13%, $\chi^2(1) = 5.67$, $p < 0.001$); one participant reported past day

Table 2
Sensitivity, specificity, and χ^2 tests of the uEtG tests with respect to past day drinking, past 3-day drinking and past 3-day binge drinking.

	Sensitivity (% of drinking detected)	Specificity (% of non-drinking detected)	χ^2	df	p
Past Day Drinking	73.91%	80.00%	18.73	1	< 0.001
Past 3-Day Drinking	43.64%	84.62%	2.94	1	0.11
Past Day Binge Drinking	83.33%	66.13%	5.67	1	< 0.001
Past 3-Day Binge Drinking	39.39%	62.86%	0.04	1	0.30

binge drinking during the TLFB interview but did not have a positive uEtG result. However, when examining the sensitivity and specificity of the uEtG test in detecting drinking within a 3-day window, performance was well below standard thresholds (past 3-day drinking: sensitivity = 43.64%, specificity = 84.62%, $\chi^2(1) = 2.94$, $p = 0.11$; past 3-day binge drinking: sensitivity = 39.39%, specificity = 62.86%, $\chi^2(1) = 0.04$, $p = 0.30$). These results suggest that when using this commercially available uEtG test with a detection cutoff of 500 ng/mL, detection of past 3-day drinking, or even past 3-day binge drinking did not exceed chance.

4. Discussion

The present study sought to test the validity of a commercially available uEtG test to detect past day drinking, past day binge drinking, past 3-day drinking, and past 3-day binge drinking in a sample of heavy drinkers. We found that while uEtG was reasonably able to detect past day alcohol use and past day binge drinking, detection of drinking and binge drinking in the past 3 days was poor. These findings were consistent with a recent study examining the utility of uEtG testing among women of childbearing age, which found poor sensitivity to detect light-to-moderate drinking beyond a 12-hour window (Graham et al., 2017). These preliminary results call into question the validity of commercially available urine EtG tests at the manufacturer recommended detection cutoffs as means of validating alcohol abstinence and binge drinking in clinical research. It is important to consider the sensitivity of detection window as the current uEtG was commercially sold to detect alcohol use in the past 80 h, yet was only accurate for detecting past 24 h' alcohol use. As false positives are common with uEtG tests (Costantino et al., 2006; Wurst et al., 2015), researchers should be aware of the limitations of urinary EtG using the manufacturer recommended detection threshold of 500 ng/ml and should not rely on commercial uEtG alone as verification of past alcohol use, particularly when using conservative detection thresholds. Breath alcohol concentrations (BrAC) should be used in conjunction with physiological biomarkers and self-report in order to accurately capture recent alcohol intake.

Several limitations from the present study must be considered. The urine EtG test used in the current study had a threshold of 500 ng/ml, which is a conservative detection threshold based on prior studies (Jatlow et al., 2014; SAMHSA, 2006; Vinikoor et al., 2018) and may be more appropriate for use in forensic settings where non-beverage alcohol use is more common. This suggests that the uEtG test could be more sensitive if a lower detection threshold was implemented. Secondly, this study only used a self-report measure of drinking (TLFB) to validate uEtG as a biomarker. Because the TLFB interview only collects information on a drinking by day basis, we could not assess the number of hours since the last drink and thus used number of days since last drink as our reference measure. Future studies should compare the commercial test with other measures of alcohol consumption, such as LC-MS/MS methods, wearable biochemical sensors (Panneer Selvam, Muthukumar, Kamakoti, & Prasad, 2016), or ecological momentary assessment self-reports. Third, this study had a small sample size collected from a convenience sample. Future studies should enroll larger samples to examine the sensitivity of point-of-care tests.

Identifying reliable and sensitive alcohol biomarkers represents an

important task to advance the field of alcoholism research and practice. Provided they are accurate, alcohol biomarkers can serve as important treatment outcomes in clinical trials and health care settings to detect, monitor, and prevent alcohol use. The present study is the first investigation of the reliability of uEtG to detect recent drinking in a clinical research sample of heavy drinking individuals using a commercially available point-of-care test. Our findings contribute to the mixed literature of the validity of EtG tests, which collectively suggest uEtG measured with commercially available assays at conservative cutoff thresholds (e.g. 500 ng/mL) are not reliable enough to serve as a primary alcohol biomarker. Urine EtG tests using lower cutoffs (100–200 ng/mL) appear to serve as more reliable predictors of heavy alcohol use (Jatlow et al., 2014; McDonnell et al., 2015), and as such, these thresholds should be used in clinical research settings seeking to use biological verification of heavy alcohol use. Continuous efforts to reach acceptable levels of sensitivity and specificity with commercial assays that are affordable, easily administered, and user-friendly hold potential to advance the measurement of alcohol intake, ultimately overcoming the pitfalls of self-report data.

Contributors

LAR designed the study and wrote the protocol. XN, DH, and LAR collected study sample and materials. ENG, XN, DH, and SB conducted statistical analyses. ENG drafted the manuscript and all authors contributed to and have approved the final manuscript.

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CRediT authorship contribution statement

Erica N. Grodin: Conceptualization, Validation, Writing - original draft. **Xuan-Thanh Nguyen:** Conceptualization, Investigation, Data curation, Writing - review & editing. **Diana Ho:** Data curation, Writing - review & editing. **Spencer Bujarski:** Conceptualization, Formal analysis. **Lara A. Ray:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

LAR has received study medication from Pfizer Medicinova and consulted for GSK and Mitsubishi Tanabe. None of the authors have conflicts of interest to disclose.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.abrep.2020.100249>.

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