Published in final edited form as: Subst Use Misuse. 2016 January 2; 51(1): 126–130. doi:10.3109/10826084.2015.1073324.

# Commercial Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) Testing is not Vulnerable to Incidental Alcohol Exposure in Pregnant Women

Steven J. Ondersma, Jessica R. Beatty, Thomas G. Rosano, Ronald C. Strickler, Amy E. Graham, and Robert J. Sokol

## **Abstract**

**Background**—Ethyl Glucoronide (EtG) and Ethyl Sulfate (EtS) have shown promise as biomarkers for alcohol and may be sensitive enough for use with pregnant women in whom even low-level alcohol use is important. However, there have been reports of over-sensitivity of EtG and EtS to incidental exposure to sources such as alcohol-based hand sanitizer. Further, few studies have evaluated these biomarkers among pregnant women, in whom the dynamics of these metabolites may differ.

**Objectives**—This study evaluated whether commercial EtG-EtS testing was vulnerable to high levels of environmental exposure to alcohol in pregnant women.

**Methods**—Two separate samples of five nurses—one pregnant and the other postpartum, all of whom reported high levels of alcohol-based hand sanitizer use—provided urine samples before and 4–8 hours after rinsing with alcohol-based mouthwash and using hand sanitizer. The five pregnant nurses provided urine samples before, during, and after an 8-hour nursing shift, during which they repeatedly cleansed with alcohol-based hand sanitizer (mean 33.8 uses). The five postpartum nurses used hand sanitizer repeatedly between baseline and follow-up urine samples.

**Results**—No urine samples were positive for EtG-EtS at baseline or follow-up, despite use of mouthwash and—in the pregnant sample—heavy use of hand sanitizer (mean of 33.8 uses) throughout the 8-hour shift.

**Conclusions/Importance**—Current, commercially available EtG-EtS testing does not appear vulnerable to even heavy exposure to incidental sources of alcohol among pregnant and postpartum women.

#### **Keywords**

ethyl glucuronide; ethyl sulfate; substance abuse detection/methods; pregnancy; a	Alcohol-Related
Neurodevelopmental Disorder	

## Introduction

Biomarkers are of interest as a supplement to self-report measures of alcohol use in research studies involving human subjects. They may have particular utility with populations in whom drinking is stigmatized or could lead to negative consequences. This is particularly true of pregnant women who under-report drinking during pregnancy (Alvik, Haldorsen, Groholt, & Lindemann, 2006; Ernhart, Morrow-Tlucak, Sokol, & Martier, 1988; Morrow-Tlucak, Ernhart, Sokol, Martier, & Ager, 1989). However, no alcohol biomarker has demonstrated sufficient validity and window of detection to earn widespread acceptance as a primary outcome measure (Allen & Litten, 2003).

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are of interest because they are direct biomarkers, meaning that they are specific analytes of alcohol metabolism and thus are only detectable as a result of alcohol exposure. Further, they are reported to remain detectable in urine for 1 – 2 days or longer (Palmer, 2009). However, reports of over-sensitivity to incidental alcohol exposure through hand sanitizer (Reisfield, Goldberger, Crews, et al., 2011), trace amounts of alcohol in some foods (Musshoff, Albermann, & Madea, 2010), and mouthwash (Costantino, Digregorio, Korn, Spayd, & Rieders, 2006; Reisfield, Goldberger, Pesce, et al., 2011) have led to suggestions in a recent SAMHSA review for more research regarding cutoff values that balance sensitivity and specificity in research contexts (Substance Abuse and Mental Health Services Administration, 2012). This 2012 SAMHSA review also noted substantial need for research regarding the role of individual factors such as gender, age, or medical condition to aid in interpretation of these biomarkers. Pregnancy, given its altered physiology, is an important example of the kind of individual factor that should be investigated, particularly given the potential negative impact of alcohol on pregnancy outcomes.

The sensitivity of EtG and EtS, although problematic in some contexts, makes them of particular interest with pregnant women (Bakhireva & Savage, 2011; Joya et al., 2012) given the potential for fetal harm from even light or moderate drinking (May et al., 2013; Sokol, Delaney-Black, & Nordstrom, 2003). Known as Fetal Alcohol Spectrum Disorders (FASD), these effects manifest as a group of conditions ranging from mild to severe, and can include physical, behavioral, and neurocognitive deficits. A study of 103 women in Sweden found that use of biomarkers, including EtG and EtS, led to significantly greater identification of at-risk women compared to self-report screening alone (Wurst et al., 2008).

Because of concerns regarding over-sensitivity, and because of the relatively greater public demand for workplace testing and forensic toxicology, commercial laboratories have implemented a range of methods for minimizing false positive results. Many now require that both EtG and EtS score above a priori cutoffs before a sample will be considered positive. For urine samples, cutoff scores as high as 500 ng/ml for EtG have been used (e.g., Abudu, 2008). Redwood Toxicology Laboratory (Santa Rosa, California) uses an algorithm requiring EtG and EtS to be above a cut score before results are reported as positive (for Redwood, those cutoffs are 100 ng/ml for EtG and 25 ng/ml for EtS; "EtG/EtS Alcohol Testing," 2011).

The present study examined the utility of this EtG-EtS algorithm for detecting alcohol use in pregnancy by evaluating its vulnerability to false positives due to incidental environmental exposure to alcohol. A commercial laboratory was used to enhance the relevance of our findings to routine practice. We chose Redwood Toxicology because of their relatively liberal criteria for reporting EtG and EtS results. We tested the Redwood Toxicology two-part algorithm with pregnant and postpartum nurses who use hand sanitizer regularly to limit the spread of diseases in health settings.

#### **Materials and Methods**

## **Participants**

Participants were ten nurses age 18 or older who were negative on the T-ACE alcohol risk screener (see below for more on the T-ACE), reported no drinking in the past week, and reported regular use of alcohol-based hand sanitizer. Five participants were currently pregnant, and five were 1–3 days postpartum (still hospitalized). All nurses were recruited through Henry Ford Health System email and newsletters, and by word of mouth. Participants received a \$50 gift card for participation.

#### Measures

Primary measures were the Redwood EtG-EtS algorithm and the T-ACE alcohol screener, a validated four-item self-report risk screener (Sokol, Martier, & Ager, 1989). Urine samples were sent to the Redwood Toxicology Laboratory, where they were tested using the EtG and EtS algorithm noted above (in which samples testing above 100 ng/ml for EtG, and also testing above 25 ng/ml for EtS, are considered positive). The T-ACE consists of four items evaluating Tolerance, concern expressed by others (Annoyed), ever feeling the need to Cut down, and use of alcohol in the morning (Eye-opener). The values of each answer for the four questions are summed to determine a final score ranging from 0 to 5. A total score of 2 or greater is considered a positive screen. Participants were also asked to report the number of hand sanitizer uses in the four days prior to testing, as well as on the day of testing.

## **Procedures**

Nurses responding to requests for volunteers were screened for eligibility by phone. Participants meeting criteria were interviewed prior to the beginning of a shift (if pregnant) or in their private hospital rooms (if postpartum), at which time they reviewed the informed consent information sheet for this anonymous study. Those who provided consent were asked to report estimated number of hand sanitizer uses in the four previous days and submitted a baseline urine sample. They then used the hospital's alcohol-based hand sanitizer (Soft'N Sure™ Foamed Antiseptic Handrub, Steris, Inc.; Mentor, OH; 62% alcohol) and rinsed for 15 seconds with an 18.9% alcohol content mouthwash (Scope® by Procter & Gamble). Participants then provided an additional urine sample 4 hours after the baseline sample was provided, and also provided information about frequency of hand sanitizer use during the preceding 4-hour period. Pregnant participants also provided an additional urine sample 8 hours after baseline, again with a record of hand sanitizer use during the past 4 hours. All procedures for this study were approved by the Institutional Review Boards of Wayne State University and the Henry Ford Health System in Detroit, MI.

## Results

Mean age of participants was 30.8 (range 24 to 37); 70% were White, 20% were Asian, and 10% were Black. None of the 25 urine samples tested positive for EtG and EtS (Table 1). Mean gestation for the pregnant nurses was 24.8 weeks (range 12 to 38); postpartum nurses gave birth at a mean of 39.6 weeks gestation (range 38 to 40).

## **Conclusions**

The key finding of this study is that the combined EtG and EtS algorithm was not vulnerable to false positive results from use of mouthwash (18.9% alcohol) and hand sanitizer (62% alcohol) in pregnancy or in the postpartum period. These results are consistent with those of a prior study showing that incidental exposure, although resulting in detectable levels of EtG, failed to result in levels above 100 ng/ml for any participants (Rosano & Lin, 2008). As noted by the SAMHSA review of alcohol biomarkers (2012), research standards—in which biomarker results are often aggregated within experimental conditions—differ from forensic standards, in which the chance of a false positive for any given individual must be negligible. That is, some degree of false positives can be tolerated in a research context if the sensitivity and overall accuracy of a test are sufficient. There remains a need for further exploration of individual cut scores or alternate algorithms (e.g., either EtG > x or EtS > y) that might balance sensitivity and specificity with these direct metabolites. EtG and EtS testing with lower limits of quantitation and without forensic algorithms is also needed.

The present findings also suggest that further research should evaluate measurement of EtG and EtS in hair in an effort to extend the detection time for ethanol use. EtG in hair offers a much greater window of detection and has emerged as a superior biomarker in some studies (Kharbouche et al., 2012; Pirro et al., 2011). However, Kronstrand et al. (Kronstrand, Brinkhagen, & Nystrom, 2012) could definitively detect EtG in the hair of only 1 of 14 women who ingested approximately one standard drink per day for 3 months. Morini and colleagues (2010) suggested that maternal ingestion of at least 30 grams of ethanol per day would be necessary for detection in hair.

This study has several strengths, including a sample with high levels of natural exposure to alcohol-based hand sanitizer, testing a commercially available method of alcohol detection via EtG-EtS, and a focus on alcohol use in pregnancy. Limitations include its small sample size and the lack of information regarding EtG and EtS separately (that is, it is not known how often one of these biomarkers may have been positive when the other was negative, since Redwood only reports values when both are positive). Future research might examine EtG and EtS in a larger and more diverse sample of pregnant/postpartum women.

There is a clear need for a sensitive and valid biomarker for alcohol use. This is particularly true given evidence of an association between Fetal Alcohol Spectrum Disorders (FASD) and ingestion of even three drinks per episode on two days per week during pregnancy (May, et al., 2013). The importance of identifying a valid biomarker for alcohol use is perhaps nowhere greater than during pregnancy, particularly with subgroups of pregnant women who

are particularly hesitant to disclose drinking behavior, or in specific geographic areas where the prevailing laws make negative consequences of disclosure especially likely.

# Acknowledgments

Funding for this research was provided by NIH grant AA020056 and by Joe Young Sr./Helene Lycaki funding from the State of Michigan. The authors gratefully acknowledge the assistance of staff at the Henry Ford Health System's (HFHS) New Center One prenatal care clinic, the HFHS nurses who participated in this study, and research assistant Shetoya Rice.

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Table 1

Hand Sanitizer (HS) Use and Ethyl Glucuronide (EtG)/Ethyl Sulfate (EtS) Results for Pregnant and Post-partum Nurses<sup>a</sup>

Pregnant	Pregnant HS total in past 4 days	Baseline EtG/Ets	HS use between sample 1 & 2	4 Hour EtG/Ets	in past 4 days Baseline EtG/Ets HS use between sample 1 & 2 4 Hour EtG/Ets HS Use between sample 2 & 3 8 Hour EtG/Ets	8 Hour EtG/Ets
1	165	ı	14	ı	20	1
2	140	ı	30	ı	18	
3	35	ı	12	ı	6	1
4	09	ı	20	ı	10	1
v	78	1	22		14	
Postpartum						
1	9	ı	3	ı		
2	0	ı	-	ı		
3	34	ı	П	ı		
4	23	ı	П	ı		
5	12	ı	6	I		

<sup>a</sup>All EtG - EtS results were negative. All participants rinsed with an 18.9% alcohol content mouthwash for 15 seconds after providing the baseline urine sample.