Technical Article

by Pamela Bean and Mary Jones

Overcoming Incidental Positives in Ethyl Glucuronide Testing—Lessons Learned

Ethyl glucuronide (EtG) is formed in the liver by conjugation of ethanol with activated glucuronic acid in a reaction that represents only a small fraction (0.02–0.06%) of the total elimination of ethanol by the human body. EtG is a unique and sensitive indicator of ethanol exposure and a very reliable indicator of alcohol use. Unlike urine or blood ethanol, which can be detected for only a few hours after an individual consumes his/her last drink, EtG remains elevated for 2–5 days after alcohol consumption has stopped.[1,2] EtG has typically been quantified by GC-MS, preferably following its extraction from urine or serum samples by solid-phase extraction techniques.[3] With this procedure, limits of detection range between 0.03 and 0.05 mg/L. In addition, utilizing LC/MS-MS, diluted urine samples can be run directly with a limit of detection of approximately 0.04 mg/L. A more recent enzyme multiplied immunoassay (EMIT) uses polyclonal antibodies to determine EtG in both serum and urine.[4]

Diagnostic performance

Several publications over the past decade have compared EtG concentrations in the urine of alcoholics with nonalcoholic controls. The most relevant study[5] describes EtG diagnostic performance in more than 400 patients and uses a receiver operating characteristic (ROC) curve analysis to distinguish between nondrinkers and individuals who remained sober for more than four days versus individuals drinking in the recent four days. Using a cutoff of 0.145 mg/L, clinical sensitivity is reported as 84% and clinical specificity as 68%. For those with a self-reported sobriety of less than 24 hr, sensitivity is reported as 91% and specificity as 77%.

Clinical benefits

In clinical practice, EtG is currently being used in the U.S. to identify alcohol consumption in impaired professionals.[6] Other alcohol markers, such as carbohydrate deficient transferrin (CDT) and the liver enzymes, have substantially lower sensitivities than EtG and are therefore not recommended for programs that require full alcohol abstinence. Physicians health programs across the nation are using the EtG test in three primary ways: 1) for "cause" testing when there is credible suspicion of alcohol use, 2) to confirm a positive urine alcohol test when alcohol use is denied, and 3) to monitor for relapses to alcohol during follow-up. When used appropriately, EtG testing alone or as a complement with self-report usually leads to significant improvements in treatment outcomes.

Perceived challenges

A recent report[7] on alcohol biomarkers released by the Substance Abuse and Mental Health Service Administration (SAMHSA) raises the question as to whether EtG is too sensitive for clinical use because it detects incidental alcohol exposure unrelated to the consumption of alcoholic beverages. Incidental use could include the utilization of ethanol-containing mouthwashes, ethanol-containing hand sanitizers, some over-the-counter cough medicines, and/or exposure to ethanol vapors in certain work areas. To date, there are only a few published reports describing the correlation of unintentional ethanol exposure and the detection of EtG in urine. For this reason, this article examines the effects of unintentional use on EtG testing after exposing two volunteers to alcohol-containing vapors and products. Most importantly, the article also describes several simple approaches to overcome the incidental EtG positive results reported previously.

Sources of interference

Mouthwash and breath sprays

One of the main sources of unintentional ethanol ingestion is the use of ethanol-containing mouthwashes and breath sprays. These products contain between 8 and 26% ethanol by weight such that a typical package of mouthwash (32 fluid oz) can have an ethanol level equivalent to up to 20 standard drinks. Ethanol is easily absorbed in the oral cavity and inevitably some will trickle down the back of the throat and could generate a positive result for EtG.

In-house data collected at U.S. Drug Testing Laboratories (USDT Laboratories, Des Plaines, IL) used two subjects (middle age, both Caucasians, male and female) who abstained from consuming alcoholic beverages during the length of these studies. Both volunteers used 20 mL of antiseptic mint mouthwash containing 21.6% ethanol once every hour for 8 hr. After exposure to the mouthwash, urine samples were collected at 2, 4, 6, 8, and 18 hr (corresponding to the first void next morning) and EtG was analyzed using the company's standard procedure on an API 2000 LC/MS/MS (**Applied Biosystems**, Foster City, CA); the limit of detection was 38.7 ng/mL. For comparison purposes, the results were normalized to a creatinine level of 100 mg/dL.

The results of these experiments show that it is possible to generate detectable concentrations of EtG in urine by using ethanol-containing mouthwash as directed on the manufacturer's label. The highest EtG concentration achieved

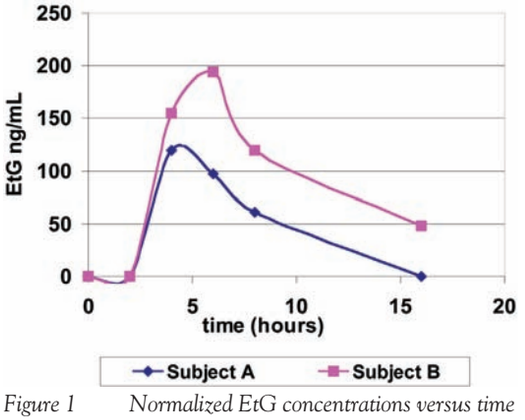

Figure 1 Normalized EtG concentrations versus time after using mouthwash.

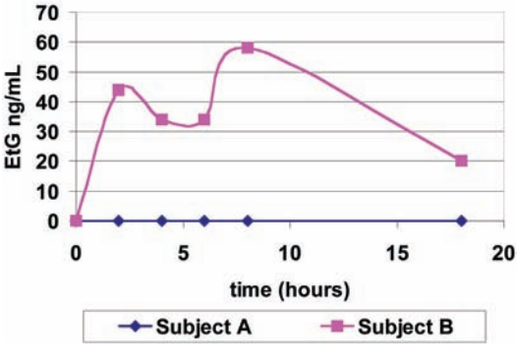

Figure 2 Normalized EtG concentrations versus time after using a hand sanitizer.

over an 8-hr period was 194 ng/mL (*Figure 1*). In a similar study[8] by a different group of scientists, nine volunteers were given a 4-oz bottle of mouthwash containing 12% ethanol and were asked to gargle with the entire bottle over a 15-min period. A total of 39 urine specimens were collected; 32 of 39 EtG values tested below 200 ng/mL, eight tested between 200 and 300 ng/mL, and one specimen tested at 345 ng/mL, with all peak values occurring at less than 12 hr after gargling. These studies show that exposure to ethanol-containing mouthwash results in incidental positives when testing for EtG in urine. However, a simple solution for subjects being monitored with the EtG test during alcohol treatment is to avoid the use of ethanol-containing mouthwash products.

Hand sanitizers

Another possible source of incidental positives for EtG testing results from the use of ethanol-containing hand sanitizers. These products contain between 60 and 65% ethanol by weight and, since ethanol is readily absorbed into the skin, a typical purse-sized package (4 fluid oz) can contain the equivalent of as much as six standard drinks.

Similar in-house experiments conducted at USDT Laboratories collected data on the same volunteers after using 0.5 g of hand sanitizer X containing 62% ethanol and massaging the gel into the hands once every hour for 8 hr. Urine samples were again obtained at 2, 4, 6, 8, and 18 hr after exposure, and EtG was analyzed as described above. The results showed no detectable concentrations of EtG in the female volunteer; the highest EtG concentration achieved in the male was 58 ng/mL (*Figure 2*). The female volunteer was then asked to use an excessive amount of hand sanitizer (2.0 g) at the same time periods, and the highest EtG concentration achieved was 799 ng/mL 3 hr after exposure to the product.

A related study[9] by a different group used another hand sanitizer (Y) at various frequencies during an 8-hr period, and the only positive specimen found was from a participant who showed 62 ng/mL of EtG after using the sanitizer every 15 min for 8 hr. These results showed that reasonable concentrations of ethanol-containing hand sanitizers elicit minute concentrations of EtG, and only heavy use of hand sanitizer X, such as the use expected in an intensive care unit environment, could lead to high EtG levels. Based on these findings, it is recommended that individuals participating in a program that requires full ethanol abstinence either choose a hand sanitizer that does not contain ethanol for their personal hygiene or use soap and water.

Inhalation of ethanol vapor

The third possible source of unintentional ethanol ingestion investigated in this study was the inhalation of ethanol vapor. Experiments in humans have shown that 55–60% of inhaled vapors are absorbed in the bloodstream. Formation of metabolites following exposure to ethanol vapor depend on several factors, including the concentration of ethanol in the air, the duration of exposure, breathing rate, absorption rate of ethanol across the lungs, and the body's elimination rate of ethanol.

Inhalation of vapor was studied at USDT Laboratories by adding 50 mL of reagent-grade ethanol to a 250-mL beaker; the beaker was swirled to promote vaporization and one deep respiration was performed by the two volunteers. The procedure was repeated every 5 min for 1 hr, and urine specimens were captured at 15 min, 1 hr, and 14 hr after fume exposure. Both subjects reported that the fumes were noxious and irritating to the throat, lungs, and eyes. The results of the testing showed that one subject had no detectable levels of EtG and another subject demonstrated a detectable level at 124 ng/mL. Based on these experiments, it seems highly unlikely that exposure to ethanol in most workplaces will provide enough vapor to trigger a positive unintentional EtG result.

Smart EtG testing

Based on the above experiments, there is no doubt that ethanol-containing products can produce small concentrations of EtG. However, very simple strategies can be used to overcome incidental positive results. For instance, it is highly recommended that recovering health professionals, who by contract have agreed on total abstinence after drug and alcohol therapy, choose alcohol-free personal hygiene products. It is also recommended that different EtG cutoffs are used to select a monitoring level that best fits the program goals for the client. The current recommended cutoffs are:

- 100/ng/mL: appropriate in zero-tolerance programs because of the restrictions placed on the clients
- 250/ng/mL: a relatively safe cutoff for most programs that can enforce limitations on use of ethanol-containing products
- 500 ng/mL: chosen to further reduce the possibility of positives being related to anything other than actual consumption of alcohol.

The establishment of a cutoff that can clearly distinguish consumption of alcoholic beverages from exposure to alcohol in other products is another venue to increase EtG diagnostic performance and clinical benefit.

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