

SENSITIVITY OF COMMERCIAL ETHYL GLUCURONIDE (ETG) TESTING IN SCREENING FOR ALCOHOL ABSTINENCE

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Abstract — The ‘80 h Ethyl Glucuronide (EtG) test’ has become an idiom of the alcohol testing community, a review of the literature shows this window of detection applies only to extreme cases. EtG testing is becoming more common as a method to test for alcohol consumption in individuals who have been ordered to abstain from alcohol consumption. We tested 19 subjects using commercially available EtG kits. All urine samples collected more than 26 h after drinking had false negative results.

INTRODUCTION

Ethyl Glucuronide test (ETG) is a non-volatile, water-soluble direct metabolite of ethanol, showing a high storage stability. It is one of the fastest emerging biomarkers for alcohol consumption and potentially offers several benefits over more established biomarkers.

A literature search about EtG reveals an extensive list of published scientific articles, dating back to the 1950s, when EtG was first isolated from rabbit’s urine (Kamil *et al.*, 1952). We focused our literature review and comments on the time-course and excretion profile of EtG after consuming alcohol.

One of the first kinetic profilings of EtG in human test subjects was published in 1997 (Schmitt *et al.*, 1997). The researchers concluded that EtG was detectable in blood serum only after alcohol consumption, that the EtG levels decline exponentially with a half life of 2 to 3 h, and that testing for EtG is restricted to a period from 6 h to more than 18 h, depending on the alcohol dose and subject’s metabolism.

Many of the published results since then are for tests performed on populations suspected of covert drinking, such as psychiatric inpatients and recovering physicians (Wurst *et al.*, 2003; Skipper *et al.*, 2004). In some of these tests patients’ actual drinking patterns are completely unknown, while in others, positive EtG results led to questioning of the patient who then admitted to alcohol consumption. Other studies deal with people who have consumed substantial amounts of alcohol, such as hospitalized alcohol withdrawal patients, but how much they drank and when they drank is unknown (Wurst *et al.*, 2002).

The effects of water-induced diuresis (i.e. dilution) and food consumption have also been documented in the published literature (Dahl *et al.*, 2002; Goll *et al.*, 2002; Stephanson *et al.*, 2002). Studies show that the intake of water prior to urine sampling results in a dramatic reduction in the EtG concentration, while expressing EtG as a ratio to creatinine is not affected by dilution.

On the whole, our literature review does convince us that EtG testing is very *specific* for alcohol. However, it

actually revealed very little quantitative information about EtG’s *sensitivity* over time and relative to the amount of alcohol consumed. Most of the research is designed to find the *true positives*, but it is not reliable for determining the rate of *false negatives*. In screening for alcohol abstinence, knowing the rate of false negatives is very important. In addition, many different limits of detection are used for differentiating between positives and negatives, and sensitivity and the window of detection was typically reported only in very general terms using phrases like ‘up to 80 h’, or ‘up to 5 days’, without the caveat that these detection windows apply only to the most extreme cases. In fact, only one published study comes close to answering the question about EtG sensitivity over time and relative to the amount of alcohol consumed (Borucki *et al.*, 2005). In this study, 17 test subjects were dosed to severely high levels in a hospital setting. For each test subject, the levels of four biomarkers (including EtG) were tested eight times over a 102-h period after drinking. Unlike most of the other research, this study used measured alcohol doses and a positive cut-off of 100 ng/ml. In the first 24 h after drinking, all EtG tests were positive. After 54.3 h, 77% of the test results were positive; while after 78.5 h, only 18% of the test results were positive.

Based on the fact that limited information was available regarding false negatives, and the fact that all research to-date was conducted in a hospital or lab setting, we decided to conduct our own small study in an office environment using commercially available test kits, just as a monitoring agency would do.

MATERIALS AND METHODS

Study design

Nineteen healthy adults participated in our study (mean age, 43 years; mean body mass index, 27 kg/m².) Test participants were recruited by word of mouth and consisted of company employees, friends, and family members. All test subjects were volunteers and fully consented to the work being done in the study and were not paid for their efforts. Each participant self-certified that they were in good general medical health, were a social drinker not dependent on alcohol and were

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Table 1. Test participant characteristics

	Dosing group			Overall
	Low	Medium	High	
Number of participants	5	7	7	19
Age, mean (SD)	43 (8)	40 (6)	45 (8)	43 (7)
Female gender, % (<i>n</i>)	20% (1)	43% (3)	29% (2)	32% (6)
Weight, mean pounds (SD)	199 (60)	172 (29)	187 (47)	185 (44)
Mean kilograms (SD)	90 (27)	78 (13)	85 (21)	84 (20)
Height, mean inches (SD)	70 (4)	69 (3)	70 (2)	69 (3)
Mean metres (SD)	1.8 (0.1)	1.7 (0.1)	1.8 (0)	1.8 (0.1)
Body mass index, mean kg/m ² (SD)	28 (5)	26 (3)	27 (6)	27 (5)

not pregnant (females). All test participants were 21 years of age or older. The alcohol monitoring systems (AMS) ethics committee did approve this study. Test participant characteristics are summarized in Table 1.

Each participant was assigned a target alcohol dose and target waiting period based on random selection of a number from a cup (no replacement). We used three different target alcohol doses (low, medium, and high) and three different target waiting periods (24, 48, and 72 h). Low, medium, and high target doses were defined as 0.25, 0.50, and 0.75 grams of ethanol per kg of body weight, respectively. (This corresponds to 1.4, 2.9, and 4.3 standard drinks, respectively, for a person weighing 79.4 kg.) The waiting period was defined as the period of time between completing alcohol consumption and providing a urine sample.

All test participants agreed to completely abstain from drinking alcohol for 5 days prior to the test, and self-reported that they indeed did so. Tests were conducted over a 13-week period, averaging 1.5 tests per week. On their scheduled day and time, each test participant selected an alcoholic beverage from among beer, wine, or 80-proof spirits. The volume of drink to be consumed was calculated based on the participant's body weight, the alcohol content of the selected beverage, and the target dose of ethanol. Drinks were generally rounded up to the nearest 29.57 ml (wine and spirits) or 354.84 ml (beer), and those who chose spirits were allowed to mix the alcohol with a clear mixer such as fruit juice or soda pop. We did not require that alcohol be consumed in a specific duration, but we did want to ensure that blood alcohol concentration (BAC) levels did not achieve dangerously high levels. Therefore, for the medium and high dose groups, a minimum drinking time was suggested to ensure drinking was stretched out over a suitable period. Overall, actual consumption durations ranged from less than 15 min to over 2 h. Participants' BACs were not measured as we were more interested in determining EtG sensitivity relative to the alcohol dose itself, not to the BAC achieved from that dose. However, because BAC does provide a reference point for many readers, we did calculate theoretical BACs for each drinking event using the Widmark formula (Anderson *et al.*, 2003). After consuming alcohol, each test participant again agreed to completely abstain from alcohol throughout their waiting period until after they provided a

urine sample. A summary of actual alcohol doses and waiting periods for test participant is shown in Table 2.

Analysis of urinary EtG

At the end of their waiting period, each test participant provided a urine sample, and samples were analysed by Northwest Toxicology Labs (NWT) in Salt Lake City, Utah. Samples were collected in an office restroom using the urine specimen collection kits provided by the lab, and they were couriered to NWT via overnight courier using the 'Express Lab Packs' provided. Due to the voluntary nature of testing, we did not directly observe the test participants when providing the sample, but we made every other effort to use the same protocol that a typical monitoring agency would use. This included filling out the chain of custody form for each specimen, ensuring at least 30 ml of sample was provided, ensuring the specimen was between 32 and 37°C after collection, and having the test participant witness sealing of the specimen container and initialing that seal. On several occasions the sample was collected after the courier's 5:30 PM pick-up time. In these cases, the sample was stored in a refrigerator (4°C) until collection the next business day. Test participants were referred to by a 4-digit ID number on all paperwork to ensure their privacy.

NWT analysed specimens only for EtG using liquid chromatography/tandem mass spectrometry (LC/MS-MS). To reduce the opportunity for false positives, the lab uses the same two-step process for EtG testing that is used for most urine tests. That is, the first test is referred to as a screening test. If the screening test is positive, then a second test is performed as a confirmation test. The second test must also be positive in order to confirm the specimen is positive for EtG. We chose a positive cut-off level of 100 ng/ml, which is the lowest (i.e. most sensitive level) offered by the lab. It should also be noted that urinary creatinine levels were not monitored. To ensure our specimens were treated the same as those delivered from any monitoring agency, NWT was not aware of the fact that we were performing a scientific study.

Test results were sent to AMS via email. The electronic report included the participant's 4-digit ID number, date that the specimen was received by the lab, date the report was created, name of the certifying scientist, screening cut-off level, and test result (positive or negative) for both the screening test and confirmation test (when applicable). AMS was billed \$35 per test.

RESULTS

Test results grouped by dosing group and waiting period are shown in Table 3. We can immediately see from these results that the only positive tests occurred at the 24-h waiting period in the medium dose and high dose groups, where 67 and 100% of the drinking episodes were confirmed, respectively.

Inversely, no positive EtG tests were reported after either a 48 or 72 h waiting period. All 11 alcohol doses with these waiting periods resulted in a false negative test, and this included doses as large as 0.85 grams of ethanol per kg of body weight, 6.4 standard drinks, and an estimated 0.109

Table 2. Alcohol doses and waiting periods

Test participant	Dosing group	Actual alcohol dose (g ethanol per kg body weight)	Actual waiting period (h)	Number of standard drinks	Duration of consumption (min)	Calculated peak BAC (g/dl)
1	Low	0.19	24	1.0	25	0.033
2	Low	0.26	24	1.4	15	0.034
3	Low	0.28	49	1.5	60	0.028
4	Low	0.25	72	2.4	30	0.033
5	Low	0.25	73	1.6	30	0.029
6	Medium	0.39	28	2.1	250	0.039
7	Medium	0.50	24	2.6	100	0.066
8	Medium	0.58	25	3.4	210	0.032
9	Medium	0.50	49	3.4	60	0.059
10	Medium	0.50	48	3.1	65	0.058
11	Medium	0.50	55	1.9	30	0.087
12	Medium	0.50	77	2.9	14	0.071
13	High	0.66	25	2.6	220	0.031
14	High	0.75	24	4.6	50	0.099
15	High	0.76	25	4.4	70	0.061
16	High	0.75	48	4.8	120	0.079
17	High	0.76	48	4.0	120	0.109
18	High	0.72	74	6.4	88	0.085
19	High	0.85	72	5.0	150	0.089

Table 3. Positive tests by dosing group and waiting period

		Waiting period		
		24 h (%)	48 h (%)	72 h (%)
Dosing Group	High	100 (3 of 3)	0 (0 of 2)	0 (0 of 2)
	Medium	67 (2 of 3)	0 (0 of 2)	0 (0 of 2)
	Low	0 (0 of 2)	0 (0 of 1)	0 (0 of 2)

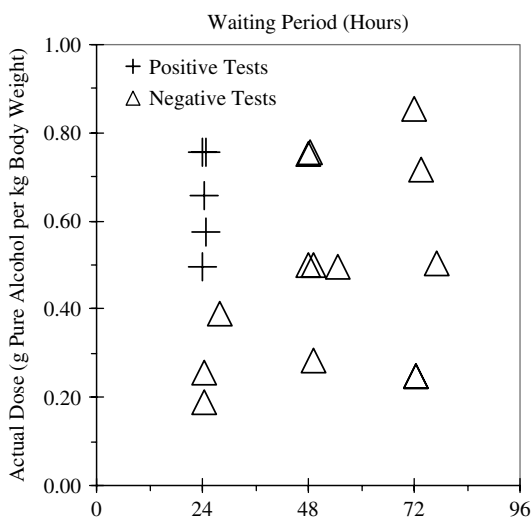


Fig. 1. Individual test results by actual dosage and actual waiting period.

BAC. For the low dose group, no tests were confirmed positive, regardless of the waiting period.

Figure 1 illustrates the results graphically, with the actual waiting period along the horizontal axis and the actual dose along the vertical axis. Plus signs represent the five episodes that were confirmed positive.

In addition, we looked at the correlation between test results (positive = 1, negative = 0) and both waiting period (h) and actual alcohol dose (g ethanol per kg body weight). As expected, there is a positive correlation between the magnitude of the alcohol dose and obtaining a positive test, but the statistical significance of this correlation for our sample is not as large as expected ($r = 0.38$, $P < 0.065$).

On the other hand, there is a strong, statistically significant, negative correlation between the waiting period and obtaining a positive test ($r = -0.63$, $P < 0.002$). In other words, the likelihood of obtaining a positive test decreases as the waiting period increases. Although this result is intuitive, what is not intuitive is that within the limits of this study, test results are much more strongly correlated to the waiting period than they are to the alcohol dose itself.

DISCUSSION

Our study has several important findings: (i) Within the limits of our study, commercially available EtG testing must be conducted every 24 hours at the 100 ng/ml level to detect approximately 2/3rd of the medium and high dose episodes; (ii) Commercially available EtG testing, even using a 100 ng/ml cut-off and 24 h waiting period, is not effective at detecting low dose alcohol consumption; and (iii) Our results are actually very consistent with what is published in the scientific literature, which shows that the '80 h EtG test' only applies to very large drinking episodes.

Although our study was small, its results are very consistent with one previously described study (Borucki *et al.*, 2005).

Both studies used measured alcohol doses and a positive cut-off of 100 ng/ml. Although that study reported positive tests beyond 24 h (77% at 54.3 h, and 18% at 78.5 h), this can be explained by the fact that the alcohol doses used in that study were a great deal higher than ours, averaging 8.9 standard drinks compared to our average of 3.1 standard drinks.

Unfortunately, very general statements have been misinterpreted by the non-scientific community, and the '80 h EtG test' has become an idiomatic phrase of practitioners in the forensic market. For example, at the time of this writing, one test laboratory's website states that EtG testing will detect 'virtually any alcohol consumption 80 h after drinking,' and that a 100 ng/ml test conducted every 80 h allows 'zero-tolerance' for alcohol consumption. Based on our literature review and small study, such claims are simply not supportable.

It is also worth noting that all of the published testing to-date has taken place in a laboratory or hospital environment. Although the impact of the environment on test results is not clear, to the best of our knowledge, our research is the first study in which testing was done in a manner comparable to that used by practitioners in the forensic marketplace. For example, urine samples were collected in an office environment using inexpensive kits and couriered overnight to the commercial testing lab. Related to this, the fact that dilution of EtG is possible by consuming large quantities of water has previously been established, and researchers have recommended that test subjects' urine creatinine levels also be monitored. We were not offered that option on the commercial tests we purchased. Additionally since our subjects were all voluntary we have no reason to suspect that there was any attempt to dilute or alter in anyway the urine samples submitted for testing.

To use commercial EtG testing for abstinence screening in reality is not practical at this time. To detect low to moderate drinking episodes, EtG testing would have to take place at less than 24 h intervals. To use greater time intervals and certainly intervals as long as 80 h would result in low to moderate level drinking episodes going completely undetected. Based on this,

perhaps a more appropriate role for periodic EtG testing is to screen for daily drinking (i.e. alcohol relapse) as opposed to screening for complete abstinence.

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