
Rapid Communication

Use of Ethanol-Based Hand Disinfectants: Source of Increased Ethyl Glucuronide Levels in Hair?

Clementine Scholz, Markus R. Baumgartner, and Milena M. Madry*

University of Zurich, Zurich Institute of Forensic Medicine, Center for Forensic Hair Analytics, Kurvenstrasse 17, 8006 Zurich, Switzerland

*Corresponding author: milena.madry@irm.uzh.ch

Received 3 July 2020; Revised 25 August 2020; Accepted 31 August 2020

Abstract

Aim: Due to the COVID-19 pandemic increasing the use of hand disinfectants, we investigated the effect of frequent use of ethanol-based hand disinfectants (EBHD) on the levels of the alcohol marker ethyl glucuronide (EtG) in hair.

Method: Hair samples were collected from 10 health professionals (8 nondrinkers, 2 rarely drinking individuals) and EtG was examined in hair.

Result: EtG (~2 pg/mg) was only detected in the hair sample of a nondrinker using EBHD 60–70 times per working day.

Conclusion: Our data provide no evidence that frequent EBHD use results in hair EtG levels above the recommended Society of Hair Testing cutoff for repeated alcohol consumption (5 pg/mg).

INTRODUCTION

Ethyl glucuronide (EtG) is a phase II metabolite of ethanol and a widely used direct marker for alcohol intake. EtG is most commonly tested in blood, urine and hair (Biondi *et al.*, 2019). The matrix of choice depends on the case-based question and time window to be examined. Whereas blood and urine are tested for the detection of short-term alcohol consumption, hair allows for the retrospective monitoring over a period of several months. Therefore, hair testing is a valuable tool in driving ability assessments, abstinence monitoring and workplace drug testing. Additionally, hair offers the advantage of time-resolved long-term monitoring by segmental hair analysis.

EtG is one of the most important diagnostic markers for assessment of alcohol consumption. According to the most recent consensus of the Society of Hair Testing (SoHT), alcohol consumption can be classified based on EtG hair concentrations in the proximal head hair segment of 3–6 cm length (SoHT, 2019). A concentration ≤ 5 pg/mg (formerly 7 pg/mg) does not contradict abstinence, concentrations > 5 pg/mg strongly suggest repeated alcohol consumption and concentrations ≥ 30 pg/mg are indicative of chronic excessive alcohol consumption.

One major challenge in biomarker testing is to avoid false negative or false positive outcomes. It has been documented that cosmetic treatment of hair (e.g. bleaching, dyeing, thermal straightening) may lead to considerable decrease of incorporated EtG (Kerekes and Yegles, 2013; Petzel-Witt *et al.*, 2018). Further attempts for the manipulation of EtG concentrations have been reported by application of so-called detox shampoos. These are promoted on internet platforms to increase EtG washout. After a single application of a detox shampoo, no notable impact on the EtG levels has been observed (Binz *et al.*, 2014). However, repetitive and prolonged incubation can decrease EtG concentrations in hair (Luginbuhl *et al.*, 2019). Misleading hair testing results can also be caused by falsely elevated concentrations after hair treatment with EtG-containing cosmetics. Several commercially available herbal hair tonics have shown to contain EtG (Arndt *et al.*, 2013) and the use of an EtG-containing hair lotion yielded EtG concentrations suggesting chronic excessive drinking (Sporkert *et al.*, 2012). In contrast, the use of ethanol-containing hair cosmetics did not indicate an increase of hair EtG levels (Martins Ferreira *et al.*, 2012). Eventually, the wide diversity of commercially available ethanol-containing medication not intended

Table 1. Data on study participants sorted by frequency of hand disinfection per working day during the past 5–6 months prior to scalp hair sample collection

Participant no., sex, age (years)	EtOH content of disinfectant (v/v)	Frequency of disinfection per working day	Surgical hand wash	Average alcohol units per month	Weight (kg)	Length of hair segment 1 (cm)	EtG in segment 1 (pg/mg)	Length of hair segment 2 (cm)	EtG in segment 2 (pg/mg)
1, F, 30	EtOH 80%	3	No	Abstinent	N/A	3	ND	3	ND
2, M, 33	EtOH 80%	5	No	Abstinent	N/A	3	ND	1	ND
3, F, 39	EtOH 80%	6–10	No	Abstinent	N/A	3	ND	3	ND
4, F, 40	EtOH 80%	8	No	4	N/A	3	ND	3	ND
5, F, 44	EtOH 70%	10–25 ^a	No	2	N/A	3	ND	3	ND
6, F, 29	EtOH 80%	15–20	No	Abstinent	N/A	3	ND	N/A	N/A
7, F, 46	EtOH 89%	10–35	No	Abstinent	N/A	3	ND	3	ND
8, F, 52	EtOH 80%	~50	No	Abstinent	86	3	ND	3	ND
9, F, 59	EtOH 80%	~50	Yes	Abstinent	53	3	ND	3	ND
10, F, 34	EtOH 80%	60–70	Yes	Abstinent	55	3	~2 ^b	2	ND

F: female; M: male; ND: not detectable; N/A: not applicable.

^a10–25 times within the past 2.5 months prior to hair sample collection and 6 to 10 times beforehand.

^bApproximate concentration as below LLOQ (2.5 pg/mg hair).

for oral intake such as mouthwashes and hand disinfectants should be considered.

The present study investigates the effect of ethanol-based hand disinfectants (EBHD) as a potential confounding factor in hair testing for EtG. The use of hand disinfectants constitutes an effective element of infection control. In particular, during the COVID-19 pandemic, EBHD are not only used by health professionals but also by the general population. Therefore, the question whether alcohol biomarkers in hair can be detected after ethanol absorption via routes other than oral is of major interest.

In fact, use of EBHD can produce positive findings for ethanol in blood (Miller *et al.*, 2006b; Miller *et al.*, 2006a) and urine (Gessner *et al.*, 2016) and EtG in urine (Rohrig *et al.*, 2006; Rosano and Lin, 2008; Reisfield *et al.*, 2011; Arndt *et al.*, 2014; Salomone *et al.*, 2018; Gessner *et al.*, 2016). To our knowledge, the incorporation of EtG into hair following frequent use of EBHD has been investigated in only one study in a controlled setting (Salomone *et al.*, 2018). One abstinent individual applied EBHD 20 times daily for four consecutive weeks. EtG concentrations in urine were above the cutoff of 100 ng/ml typically considered for clinical and forensic analyses. Concentrations in head, chest and beard hair have been reported as <7 pg/mg, which was the lower limit of quantification (LLOQ) of the analytical method.

The aim of the present study was to assess in a larger population of health professionals if continuous frequent exposure to EBHD under real working conditions can result in a positive hair test for EtG.

METHODS

Study design

Study volunteers were recruited from employees in hospitals and other medical institutions. Inclusion criteria were the use of EBHD in occupational settings during the past 5–6 months prior to hair sample collection, whereas disinfection should be performed at least three times per working day with at least four working days per week. Participants with alcohol abstinence or only rare alcohol consumption within the last 5–6 months prior to hair sample collection were included. An exclusion criterion was hair treatment such as permanent coloring, bleaching or thermal straightening of at least the proximal 3 cm. Hair samples were collected once; however, a longitudinal time-resolved monitoring of alcohol abstinence or alcohol consumption was achieved by the analysis of two hair segments. Detailed information on type of disinfectant, frequency of hand disinfectant use, surgical hand disinfection and alcohol consumption habit was obtained via written questionnaires. One alcohol unit was defined as a standard drink of beer (0.3 l containing 5%, v/v, ethanol) or wine (0.1 l containing 12%, v/v, ethanol), which is equivalent to ~12 and 9.6 g ethanol per unit, respectively. Upon request, we were officially informed that approval of the Cantonal Ethics Committee of Zurich is not required for this study setting (Req-2020-00490).

Hair sample collection

Scalp hair was collected from the occipital or vertex region by cutting a hair lock as close as possible to the skin. All samples were wrapped in aluminum foil and stored under dry conditions at room temperature in the dark until analysis according to SoHT guidelines (Cooper *et al.*, 2012).

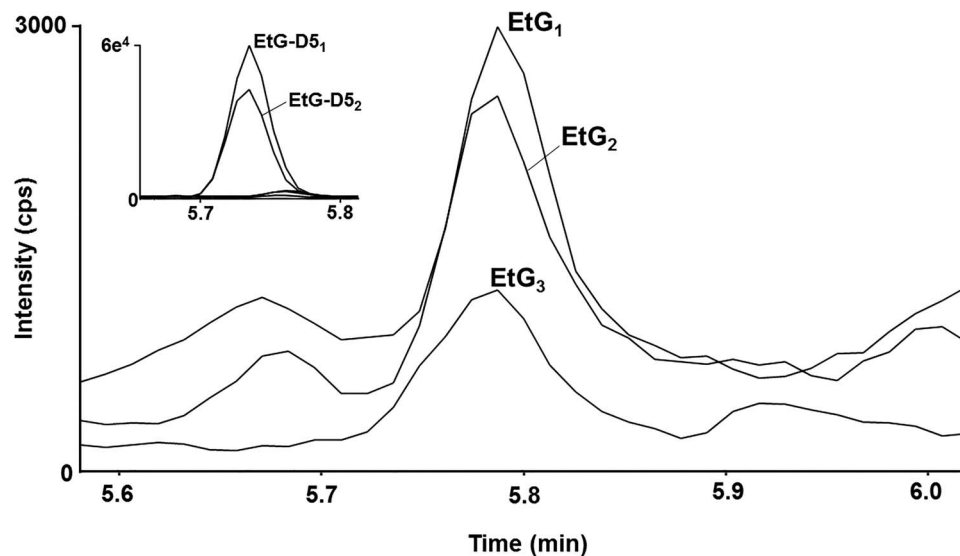


Fig. 1. Extracted ion chromatogram for EtG in the hair sample (proximal 3-cm segment) of participant no. 10. Transitions for EtG and the internal standard EtG-D5 were 221 → 75 (EtG₁), 221 → 85 (EtG₂), 221 → 55 (EtG₃) and 226 → 75 (EtG-D5₁), 226 → 85 (EtG-D5₂), respectively.

HAIR SAMPLE ANALYSIS

Hair analysis was performed in two segments: the proximal 3 cm and the subsequent 1–3 cm depending on hair length. Only hair segments without cosmetic treatment were considered for analysis. The totally tested hair segment length of 4 and 6 cm is in line with SoHT recommendations (SoHT, 2019).

EtG-free head hair collected from nondrinkers was used for calibrators and controls. Hair samples were prepared according to a previously published validated procedure (Binz *et al.*, 2014). Briefly, hair samples were successively washed by shaking with water and acetone. Samples were dried and chopped into snippets. Approximately 20 mg of snippets were pulverized. The internal standard solution was added to each sample; EtG was extracted with water under shaking in a ball mill. After centrifugation, solid phase extraction was performed. The eluate was evaporated under nitrogen. After reconstitution, the sample was injected into the liquid chromatography–tandem mass spectrometry system. The limit of detection (LOD) and LLOQ of the analytical method were 1 and 2.5 pg/mg, respectively.

RESULTS

Ten health professionals (nine female and one male) using EBHD on a daily basis could be recruited for the study. The frequency of EBHD use varied between 3 and 70 times per working day during the past 5–6 months prior to hair sample collection (Table 1). Six participants used EBHD >10 times per working day. Three participants reported very frequent use of ~50–70 times per working day; among these were two who performed surgical hand disinfection regularly (no. 9 and 10). This process includes rubbing of the disinfectant onto both hands and forearms up to 10–15 cm above the elbows. During application, the skin surfaces must be kept wet for at least 2 minutes. The ethanol content of the applied disinfectants ranged from 70 to 89% (v/v) (Table 1). Eight participants were nondrinkers for at least 5–6 months prior to hair sample collection. Two participants (no. 4 and 5) consumed on average 2–4 alcohol units per month during the last 5–6 months, respectively (Table 1). Only hair segments without cosmetic treatment were submitted for analysis. In the case of nine

head hair samples, additionally a subsequent segment with a length of 1–3 cm was tested (Table 1).

Hair samples negative for EtG

EtG was not detected in hair samples from participant no. 1–9 with EBHD use of 3 to ~50 times per working day (Table 1) applying a highly sensitive analytical method with an LOD of 1 pg/mg hair.

Hair sample positive for EtG

The proximal 3-cm hair segment of participant no. 10 was positive for EtG. Figure 1 shows the extracted ion chromatogram of the three transitions of EtG detected in the hair sample. The EtG concentration was ~2 pg/mg, which is above the LOD but below the LLOQ of our analytical method. EtG was not detected in the subsequent segment with 2 cm length. Participant no. 10 was female, nondrinker for many years and surgeon using EBHD 60–70 times daily at five to six working days per week including surgical hand disinfection.

DISCUSSION

All hair samples were collected from health professionals with frequent EBHD use in an occupational setting. The herein tested proximal 3 cm and subsequent 1–3-cm hair segments reflected a time frame of continuous EBHD use. Assuming an average head hair growth rate of 1 cm per month, the tested segments monitored ~4–6 months prior to hair sample collection. In a previous controlled study, tested hair segments covered only partly the time period of EBHD use (Salomone *et al.*, 2018). Consequently, the proportion of hair reflecting the time of EBHD use was diluted by hair reflecting the time without EBHD exposure. Thus, the possibility of EtG detection was lowered. One should also keep in mind that it takes ~10–14 days for an incorporated substance to reach the skin surface with the growing hair.

Our results provide no evidence that EtG is incorporated into hair in relevant amounts when EBHD are used frequently on a daily basis as EtG was not detected in 18 out of 19 tested hair segments. Even,

frequent EBHD use and sporadic drinking of two to four standard drinks per month (participant no. 4 and 5) did not yield detectable EtG levels.

However, EtG was detected in the proximal 3-cm segment of participant no. 10. This participant reported the most frequent EBHD use in our study including surgical hand disinfection, which consists of excessive application of hand rub on a large skin surface for several minutes. Generally, application of alcohol-based hand rubs cannot only entail dermal absorption; considerable amounts of volatilized ethanol can also be inhaled leading to pulmonary absorption. In fact, researchers postulated that ethanol from hand sanitizers is predominantly absorbed via inhalation rather than across the skin (Arndt *et al.*, 2014; Skipper *et al.*, 2009; Brewer and Streef, 2020). Hence, the frequent EBHD use of 60–70 times per working day including surgical hand disinfection may have led to EtG detection. The investigation whether skin or lungs is the pathway of ethanol absorption was beyond the scope of our study. Other explanations could be frequent use of ethanol-containing products or pharmaceuticals; however, this has been excluded. It should be mentioned that unreported rare alcohol intake cannot be entirely excluded.

EtG was not detected in the distal 2-cm segment of participant no. 10. This negative finding is in agreement with the self-reported alcohol abstinence. A potential low-level EtG incorporation into the distal hair segment due to intense EBHD use could have been washed out during personal hair hygiene. Such wash-out effects have been described in literature (Cooper *et al.*, 2012).

Overall, our findings are in line with those reported from a previous controlled study (Salomone *et al.*, 2018). Specifically, there is no evidence for obtaining an EtG hair concentration above the current SoHT cutoff for repeated alcohol consumption (5 pg/mg).

In cases in which distinction between ‘EtG detected below cutoff’ versus ‘EtG not detected’ is requested, a highly sensitive analytical method must be applied. The data suggest that bias in hair testing outcomes arising from frequent hand disinfection is negligible; however, it should be considered in the interpretation of cases with intense EBHD exposure (>50 disinfections per day). Particularly, individuals with altered ethanol metabolism or elimination due to genetic deficiencies or decreased kidney function may be more prone to have EtG detected in their hair sample.

Our preliminary findings should be confirmed in a larger population including more female and male participants with surgical hand disinfection. More potentially misleading factors such as use of other ethanol-containing products not intended for oral intake should be investigated in combination with frequent EBHD use.

CONCLUSION

Our study outcomes extend the current knowledge as the tested hair samples derive from health professionals under real working conditions. The results indicate that frequent EBHD use by nondrinkers is highly unlikely to produce a hair EtG concentration above the SoHT cutoff for repeated alcohol consumption. Therefore, alcohol abstinence testing by hair analysis is a valid tool despite exposure to disinfectants.

ACKNOWLEDGEMENTS

The authors would like to thank all study participants. Further, the authors express their gratitude to Emma Louise Kessler, MD for

the generous legacy she donated to the Zurich Institute of Forensic Medicine at the University of Zurich, Switzerland for research purposes.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

- Arndt T, Schrofel S, Gussregen B, *et al.* (2014) Inhalation but not transdermal resorption of hand sanitizer ethanol causes positive ethyl glucuronide findings in urine. *Forensic Sci Int* 237:126–30.
- Arndt T, Schrofel S, Stemmerich K. (2013) Ethyl glucuronide identified in commercial hair tonics. *Forensic Sci Int* 231:195–8.
- Binz TM, Baumgartner MR, Kraemer T. (2014) The influence of cleansing shampoos on ethyl glucuronide concentration in hair analyzed with an optimized and validated LC-MS/MS method. *Forensic Sci Int* 244: 20–4.
- Biondi A, Freni F, Carelli C, *et al.* (2019) Ethyl glucuronide hair testing: a review. *Forensic Sci Int* 300:106–19.
- Brewer C, Streef E. (2020) Is alcohol in hand sanitizers absorbed through the skin or lungs? Implications for disulfiram treatment. *Alcohol Alcohol* 55:354–6.
- Cooper G, Kronstrand R, Kintz P. (2012) Society of Hair Testing guidelines for drug testing in hair. *Forensic Sci Int* 218:20–4.
- Gessner S, Below E, Diedrich S, *et al.* (2016) Ethanol and ethyl glucuronide urine concentrations after ethanol-based hand antisepsis with and without permitted alcohol consumption. *Am J Infect Control* 44:999–1003.
- Kerekes I, Yegles M. (2013) Coloring, bleaching, and perming: influence on EtG content in hair. *Ther Drug Monit* 35:527–9.
- Luginbuhl M, Bekaert B, Suesse S, *et al.* (2019) Detox shampoos for EtG and FAEE in hair - results from in vitro experiments. *Drug Test Anal* 11: 870–7.
- Martins Ferreira L, Binz T, Yegles M. (2012) The influence of ethanol containing cosmetics on ethyl glucuronide concentration in hair. *Forensic Sci Int* 218:123–5.
- Miller MA, Rosin A, Crystal CS. (2006a) Alcohol-based hand sanitizer: can frequent use cause an elevated blood alcohol level? *Am J Infect Control* 34:150–1.
- Miller MA, Rosin A, Levisky ME, *et al.* (2006b) Does the clinical use of ethanol-based hand sanitizer elevate blood alcohol levels? A prospective study. *Am J Emerg Med* 24:815–7.
- Petzel-Witt S, Pogoda W, Wunder C, *et al.* (2018) Influence of bleaching and coloring on ethyl glucuronide content in human hair. *Drug Test Anal* 10:177–83.
- Reisfield GM, Goldberger BA, Crews BO, *et al.* (2011) Ethyl glucuronide, ethyl sulfate, and ethanol in urine after sustained exposure to an ethanol-based hand sanitizer. *J Anal Toxicol* 35:85–91.
- Rohrig TP, Huber C, Goodson L, *et al.* (2006) Detection of ethylglucuronide in urine following the application of germ-X. *J Anal Toxicol* 30:703–4.
- Rosano TG, Lin J. (2008) Ethyl glucuronide excretion in humans following oral administration of and dermal exposure to ethanol. *J Anal Toxicol* 32:594–600.
- Salomone A, Bozzo A, Di Corcia D, *et al.* (2018) Occupational exposure to alcohol-based hand sanitizers: the diagnostic role of alcohol biomarkers in hair. *J Anal Toxicol* 42:157–62.
- Skipper GE, Wurst F, Weinmann W, *et al.* (2009) Ethanol-based hand sanitizing gel vapor causes positive alcohol marker, ethylglucuronide, and positive breathalyzer. *J Addict Med* 3:1–5.
- SOHT. 2019. Society of Hair Testing: Consensus for the use of alcohol markers in hair for supporting the assessment of abstinence and chronic alcohol consumption. [Online]. https://www.soht.org/images/pdf/Revisio_n_2019_Alcoholmarkers.pdf (22 June 2020, date last accessed).
- Sporkert F, Kharbouche H, Augsburg MP, *et al.* (2012) Positive EtG findings in hair as a result of a cosmetic treatment. *Forensic Sci Int* 218:97–100.