Ethyl glucuronide in hair and fingernails as a long-term alcohol biomarker

Lisa Berger1, Michael Fendrich1, Joseph Jones2, Daniel Fuhrmann1, Charles Plate2 & Douglas Lewis2

Center for Applied Behavioral Health Research, University of Wisconsin-Milwaukee, Milwaukee, WI, USA1 and United States Drug Testing Laboratories, Inc., Des Plaines, IL, USA2

ABSTRACT

Aims This study aimed to evaluate the performance of ethyl glucuronide (EtG) in hair and fingernails as a long-term alcohol biomarker. Design Cross-sectional survey with probability sampling. Setting Midwestern United States. Participants Participants were 606 undergraduate college students between the ages of 18 and 25 years at the time of selection for potential study participation. Measurements EtG concentrations in hair and fingernails were measured by liquid chromatography-tandem mass spectrometry at three thresholds [30 picograms (pg) per milligram (mg); 20 pg/mg; and 8 pg/mg]. Any weekly alcohol use, increasing-risk drinking and high-risk drinking on average during the past 12 weeks was assessed by participant interview using the time-line follow-back method. Findings In both hair and fingernails at all three EtG thresholds, sensitivity was greatest for the high-risk drinking group [hair: 0.43, confidence interval (CI) = 0.17, 0.69 at 30 pg/mg, 0.71, CI = 0.47, 0.95 at 20 pg/mg; 0.93, CI = 0.79, 1.00 at 8 pg/mg; fingernails: 1.00, CI = 1.00–1.00 at 30, 20 and 8 pg/mg] and specificity was greatest for any alcohol use (hair: 1.00, CI = 1.00, 1.00 at 30 and 20 pg/mg; 0.97, CI = 0.92–0.99 at 8 pg/mg; fingernails: 1.00, CI = 1.00–1.00 at 30, 20 and 8 pg/mg). Areas under the receiver operating characteristic curves were significantly higher for EtG concentration in fingernails than hair for any weekly alcohol use (P = 0.02, DeLong test, two-tailed) and increasing-risk drinking (P = 0.02, DeLong test, two-tailed). Conclusions Ethyl glucuronide, especially in fingernails, may have potential as a quantitative indicator of alcohol use.

Keywords Alcohol biomarkers, alcohol drinking patterns, ethyl glucuronide, fingernails, hair.

INTRODUCTION

Alcohol biomarkers are an objective measure of alcohol exposure or alcohol use that can augment self-report and clinical history [1,2]. Older alcohol biomarkers involve indirect detection through such markers as gamma-glutamyltransferase and carbohydrate-deficient transferrin, which can be elevated as the result of large amounts of ingested alcohol or due to a number of alcohol-independent (i.e. unrelated) medical conditions [2,3]. Newer alcohol biomarkers involve direct detection through such markers as ethyl sulphate and ethyl glucuronide (EtG), which respond only to alcohol [2]. Currently, there is a need for more sensitive, across a range of alcohol use, and specific, responding to alcohol only, biomarkers that are relatively easy to measure and interpret while still being affordable [4]. EtG in hair and fingernails are potential options that may meet these specifications.

EtG is a minor metabolite of ethanol produced by conjugation pathways [1]. In urine, EtG can be detected for 24 hours or more after one or two alcoholic beverages and for as long as 2–4 days after heavier use [1]. Hair differs from urine and other specimens such as blood [2] because of its ability to serve as a long-term storage vessel of foreign substances [5]. In addition, unlike hair concentration for other drugs of abuse, EtG concentration in hair is not affected by hair color.
Nails (specifically toenails) have also been found comparable and in some cases superior to hair in mean concentrations of some drugs of abuse [8]. Ethyl glucuronide testing, when used in conjunction with alcohol use self-report (the current gold standard) [4], could have the following clinical (non-forensic) alcohol testing applications in which abstinence monitoring may occur [2]: outpatient clinical research trials; treatment programs; and organ transplant care. EtG levels, however, may vary among individuals [9], and factors that may contribute to such variability include but are not necessarily limited to gender, age and ethnic group [3]. In addition, EtG levels in both hair and nails may be affected by exposure to ethanol-containing products such as mouthwashes and hand sanitizers; therefore, an awareness of such exposures and their management may be important [1]. Finally, alcohol biomarkers in general, and in particular EtG because of the potential for extraneous exposures, should not be used as the only determinant of alcohol use, including potentially problematic use [2].

The research on EtG as an alcohol biomarker is growing. Initial studies examined EtG in urine [10], and based on these studies, EtG in urine is currently used as an abstinence monitoring tool in clinical and criminal justice settings [2]. Several studies have also examined EtG in hair, including a recent study by Lees and colleagues [11], which concluded that EtG in hair could be used to qualitatively indicate any alcohol use in the past 3 months. We identified only two published studies that have examined EtG in nails (specifically fingernails) as a long-term alcohol biomarker [12,13]. The first of these two studies, both of which were conducted by Morini and colleagues, focused on the validation of the EtG nail assay [12], and the second study, which compared both maternal nails and hair as well as neonatal meconium, focused on prenatal alcohol use exposure [13].

Although studies have included one or both matrices, the present study is the first, to our knowledge, to have examined EtG in both hair and fingernails among the same set of respondents from a more generalizable sample engaged in a range of alcohol use behaviors. Using a probability sample of college students, the present study evaluated the performance of EtG in hair and fingernails as a long-term biomarker of typical alcohol use. Specifically, we were interested in whether or not EtG was simply a qualitative indicator of any alcohol use, or if it had an ability to detect varying levels of use. We were also interested in determining whether or not EtG performance varied according to the biological matrix utilized (hair versus fingernails) or by respondent demographic/socio-demographic characteristics.

## METHODS

### Sample

The study employed a cross-sectional survey design. The study sampling frame consisted of undergraduate college students aged 18–25 at a large, public urban university located in the Midwestern part of the United States. The rationale for a college student sample was that college students engage in a range of alcohol use behaviors from no use to heavy use [14]. Based on probability sampling 1200 students were selected, of whom 606 students, 61.6% female, 81.8% white and average age 21.5 [standard deviation (SD) = 1.7] years, participated in the study during the summer and Fall of 2010 (based on a power analysis, a sample size of 600 was needed). After accounting for ineligible students (n = 78), the overall study response rate [15] was 54%.

Students were first contacted by a mailed advance letter [16], after which trained interviewers contacted students by telephone and scheduled the study interview with interested students. E-mail or text-messaging was used to contact students when students were not available by telephone. Most of the interviews were conducted in private offices on campus. Students were interviewed about their past 90-day alcohol use and then completed self-report measures via a web-based computerized self-administered interview. To validate reported alcohol use, students were asked to provide up to three collateral contacts who could verify information about their alcohol use. Thereafter, interviewers collected hair and fingernail samples from students for EtG detection. Hair was cut from the base of the scalp and was approximately the diameter of a number two pencil. Students were provided with nail clippers and were asked to provide up to 10 of their own fingernail clippings. A total of 547 students provided a usable hair sample, weight ≥5 mg, while 506 students provided a usable fingernail sample, weight ≥5 mg.

Students were compensated $25 for completing the 50-minute study interview and self-report questionnaires, and $5 for each biological specimen provided (up to $35). The study was approved by the Western Institutional Review Board and the Social and Behavioral Sciences Institutional Review Board at the University of Wisconsin.

### Measures

**Ethyl glucuronide**

Hair and fingernail samples were analyzed for EtG at United States Drug Testing Laboratories (USDTL), Des Plaines, IL, USA using liquid chromatography–tandem mass spectrometry. USDTL personnel were blinded to participant alcohol use. Sample preparation and
instrumentation methods were as published, with a 2 pg/mg limit of detection and an 8 pg/mg limit of quantitation, and the method was linear up to 2000 pg/mg [17].

**Alcohol use**

Past 90-day alcohol consumption in US standard drink units (14 g) [18] was assessed via retrospective, computerized interview using the time-line follow-back (TLFB) method [19,20]. The total number of standard drinks consumed by each participant each day within the past 84 days of the 90-day recall period was divided by 12 (the number of whole weeks in a 90-day period) in order to produce an average of standard drinks consumed each week. To maximize the accuracy of self-report using the TLFB several administration procedures were used, including the following: (i) instructing participants on the definition of a US standard drink by providing participants with a detailed reference guide as to the number of standard drinks contained in typical alcoholic beverage containers, including those often used by college students (e.g. beer pong cup); (ii) using recall aids whereby local events and participant personal events were inserted into the TLFB calendar to help participants recall their daily alcohol use; (iii) instructing participants to think about regular drinking patterns they may have to help recall their drinking; and (iv) encouraging participants to be accurate, to take their time, and that their information would remain confidential [21,22].

**Hair chemical treatment**

Participants were asked whether or not they had bleached, dyed (including highlights), permed or chemically straightened their hair either at home or by a professional in the past 90 days, as hair chemical treatments may lead to degradation or removal of EtG in hair [23].

**Analyses**

EtG hair and fingernail concentrations at three different thresholds were compared to the following past 84-day weekly alcohol consumption averages: any alcohol use (≥0 standard drinks/week); increasing risk drinking (≥15 standard drinks/week); and high-risk drinking (≥30 standard drinks/week). These categories are based, in part, on US drinking definitions [18,24] and allowed us to test and compare both EtG hair and EtG fingernail concentrations as long-term alcohol biomarkers (up to 3 months) based on typical weekly patterns of drinking. In hair, the three different EtG thresholds for a positive test were: 30 pg/mg, which according to the Society of Hair Testing strongly suggests chronic excessive alcohol use [25]; a USDTL laboratory standard of 20 pg/mg; and 8 pg/mg, the limit of quantitation. For comparison purposes, the same three EtG thresholds were also used in fingernails.

The sensitivity and specificity of both EtG hair and fingernail concentrations at the above-indicated thresholds were calculated, as were the positive and negative predictive values in relation to each of the three weekly alcohol consumption averages. Sensitivity is the proportion of participants meeting the specific alcohol use criterion who had a positive EtG test. Specificity is the proportion of participants not meeting the alcohol use criterion who had a negative EtG test. Positive predictive value is the proportion of true positive EtG tests out of both correctly classified positive and incorrectly classified positive tests. Negative predictive value is the proportion of true negative EtG tests out of both correctly classified negative and incorrectly classified negative tests. In addition, areas under the receiver operating characteristic (AuROC) curves were used to determine the ability of both EtG hair and fingernail concentrations to correctly classify participants as those meeting or not meeting the specific alcohol use criterion. AuROC curve analysis was also used to explore thresholds that resulted in higher combined values of sensitivity and specificity than the a priori thresholds of 8 pg/mg, 20 pg/mg and 30 pg/mg. Finally, logistic regression analysis was used to investigate whether or not demographic/socio-demographic factors such as gender, race/ethnicity, age, chemical treatment of hair, number of drinking days and number of heavy drinking days in the past 12 weeks (defined as ≥5 US standard drinks per day for men and ≥4 US standard drinks per day for women [18,24]) were associated with EtG hair and fingernail incorrect tests. The final sample analyzed consisted of 447 cases with sufficient testable EtG hair and fingernail sample weight (≥5 mg) and complete TLFB data. In addition, hair samples were 1.5-inch (3.8 cm) proximal segments, which account for at least 3 months of hair growth [26], and included chemically treated hair. All study analyses were performed using SAS/STAT software, version 9.3, using PROC FREQ for sensitivity, specificity, positive predictive and negative predictive values and PROC LOGISTIC for the AuROC curves and logistic regression models [27].

**RESULTS**

Of the 447 participants in the analysis sample, 63.5% were female, 83.4% were white, and on average 21.4 (SD = 1.6) years old. The majority had some weekly alcohol use (91.9%, n = 411; 8.1% abstainers, n = 36) during the past 12 weeks, while smaller proportions of the total were weekly increasing-risk drinkers (14.5%, n = 65) and weekly high-risk drinkers (3.1%, n = 14). EtG concentrations in hair ranged from 0 to 180.50 pg/mg.
and in fingernails from 0 to 397.08 pg/mg. Pearson’s correlations between average number of standard drinks consumed each week during the past 12 weeks and EtG concentrations were 0.53 (95% confidence interval (CI) = 0.46, 0.59, \( P < 0.001 \)) in hair and 0.57 (95% CI = 0.50, 0.63, \( P < 0.001 \)) in fingernails; these two correlations were not statistically different from one another (\( P = 0.60 \) using the Hotelling/Williams test, two-tailed).

Pearson’s correlation between EtG concentrations in hair and fingernails was 0.59 (95% CI = 0.53, 0.65, \( P < 0.001 \)).

Table 1a presents the EtG sensitivity and specificity calculations. In hair, sensitivity was greatest at 0.93 at the EtG threshold of 8 pg/mg for the high-risk drinking group. The corresponding specificity was 0.71, which was the lowest specificity for hair at all three EtG thresholds among the three drinking groups. In fingernails, sensitivity was greatest at 1.00 at all three EtG thresholds for the high-risk drinking group. The corresponding specificity values ranged from 0.64–0.72.

Table 2a presents the positive predictive value (PPV) and negative predictive value (NPV) calculations. In hair, a positive EtG test at all three thresholds indicated some weekly alcohol use (PPV = 0.99–1.00), whereas a negative EtG test at all three thresholds was best for increasing-risk and high-risk drinkers (NPV = 0.89–1.00 or 0–11% chance of being classified incorrectly as a negative test). Similarly, in fingernails, a positive EtG test at all three thresholds indicated some weekly alcohol use (PPV = 1.00), whereas a negative EtG test at all three thresholds was best for increasing-risk and high-risk drinkers (NPV = 0.96–1.00 or 0–4% chance of being classified incorrectly as a negative test).

AuROC curves for EtG concentrations in both hair and fingernails were performed in relation to each alcohol use criterion. In hair, the AuROC curves were: 0.66, 95% CI = 0.58, 0.73, \( P < 0.01 \) for any alcohol use; 0.76, CI = 0.69, 0.83, \( P < 0.001 \) for increasing-risk drinking; and 0.88, CI = 0.79, 0.98, \( P < 0.001 \) for high-risk drinking. The EtG levels in hair that provided the best compromise between sensitivity and specificity for each alcohol use criterion were: 8 pg/mg for any alcohol use [0.34 sensitivity (Sn); 0.97 specificity (Sp)]; 17 pg/mg for increasing-risk drinking (0.54 Sn; 0.91 Sp); and 17 pg/mg for high-risk drinking (0.86 Sn; 0.87 Sp). In fingernails, the AuROC curves were: 0.71, CI = 0.65, 0.77, \( P < 0.001 \) for any alcohol use; 0.84, CI = 0.78, 0.90, \( P < 0.001 \) for increasing-risk drinking; and 0.94, CI = 0.91, 0.97, \( P < 0.001 \) for high-risk drinking. The EtG levels in fingernails that provided the best compromise between sensitivity and specificity for each alcohol use criterion were: 17 pg/mg for any alcohol use; 17 pg/mg for increasing-risk drinking; and 17 pg/mg for high-risk drinking.

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Table 1a Sensitivity and specificity of ethyl glucuronide (EtG) in hair to detect alcohol use (n = 447).

|                  | Any alcohol use (n = 411) | Increasing-risk drinking (n = 65) | High-risk drinking (n = 14) |
|------------------|---------------------------|----------------------------------|----------------------------|
| EtG threshold    | 8 pg/mg                   | 8 pg/mg                          | 8 pg/mg                    |
| Sensitivity      | 0.10                      | 0.34                             | 0.43                       |
| 95% confidence interval | 0.07–0.13                | 0.22–0.45                        | 0.17–0.69                  |
| Specificity      | 1.00                      | 0.96                             | 0.92                       |
| 95% confidence interval | 1.00–1.00                | 0.93–0.98                        | 0.90–0.95                  |

Table 1b Sensitivity and specificity of ethyl glucuronide (EtG) in fingernails to detect alcohol use (n = 447).

|                  | Any alcohol use (n = 411) | Increasing-risk drinking (n = 65) | High-risk drinking (n = 14) |
|------------------|---------------------------|----------------------------------|----------------------------|
| EtG threshold    | 8 pg/mg                   | 8 pg/mg                          | 8 pg/mg                    |
| Sensitivity      | 0.33                      | 0.82                             | 1.00                       |
| 95% confidence interval | 0.28–0.37                | 0.72–0.91                        | 1.00–1.00                  |
| Specificity      | 1.00                      | 0.79                             | 0.72                       |
| 95% confidence interval | 1.00–1.00                | 0.75–0.83                        | 0.68–0.77                  |
use criterion were: 8 pg/mg for any alcohol use (0.42 Sn; 1.00 Sp); 37 pg/mg for increasing-risk drinking (0.80 Sn; 0.82 Sp); and 56 pg/mg for high-risk drinking (1.00 Sn; 0.82 Sp). The AuROC values for hair and fingernail EtG concentrations were significantly different for any alcohol use (P = 0.02, DeLong test, two-tailed) and increasing-risk drinking (P = 0.02, DeLong test, two-tailed), but were not significantly different for high-risk drinking (P = 0.15, DeLong test, two-tailed).

Finally, given the higher specificity but lower sensitivity of the EtG hair test for both the increasing-risk and high-risk drinking groups, logistic regression analysis was used to investigate demographic/socio-demographic differences between those participants who were classified incorrectly as positive versus classified correctly as negative on the EtG fingernail test for increasing-risk drinking at all three thresholds. Total number of drinking days and total number of heavy drinking days in the past 12 weeks were both associated significantly with participants being classified incorrectly as positive (see Table 3). That is, for every 1-day increase in the number of drinking days in the past 12 weeks, the odds of being classified incorrectly as positive versus classified correctly as negative on the EtG fingernail test increased by 3% for all three thresholds. Similarly, for every 1-day increase in the number of heavy drinking days during the past 12 weeks, the odds of being classified incorrectly as positive versus classified correctly as negative increased by 17%.

**Table 2a** Positive predictive and negative predictive values of ethyl glucuronide (EtG) in hair to detect alcohol use (n = 447).

|                  | Any alcohol use | Increasing-risk drinking | High-risk drinking |
|------------------|-----------------|--------------------------|--------------------|
|                  | >0 standard drinks/week (n = 411) | ≥15 standard drinks/week (n = 65) | ≥30 standard drinks/week (n = 14) |
|                  | 30 pg/mg | 20 pg/mg | 8 pg/mg | 30 pg/mg | 20 pg/mg | 8 pg/mg | 30 pg/mg | 20 pg/mg | 8 pg/mg |
| Positive predictive value | 1.00 | 1.00 | 0.99 | 0.56 | 0.55 | 0.32 | 0.15 | 0.18 | 0.09 |
| Negative predictive value | 0.09 | 0.09 | 0.11 | 0.89 | 0.91 | 0.93 | 0.98 | 0.99 | 1.00 |

**Table 2b** Positive predictive and negative predictive values of ethyl glucuronide (EtG) in fingernails to detect alcohol use (n = 447).

|                  | Any alcohol use | Increasing-risk drinking | High-risk drinking |
|------------------|-----------------|--------------------------|--------------------|
|                  | >0 standard drinks/week (n = 411) | ≥15 standard drinks/week (n = 65) | ≥30 standard drinks/week (n = 14) |
|                  | 30 pg/mg | 20 pg/mg | 8 pg/mg | 30 pg/mg | 20 pg/mg | 8 pg/mg | 30 pg/mg | 20 pg/mg | 8 pg/mg |
| Positive predictive value | 1.00 | 1.00 | 1.00 | 0.40 | 0.36 | 0.33 | 0.10 | 0.09 | 0.08 |
| Negative predictive value | 0.12 | 0.12 | 0.13 | 0.96 | 0.97 | 0.97 | 1.00 | 1.00 | 1.00 |

**DISCUSSION**

The results of this study support EtG as a qualitative indicator of any alcohol use in the past 12 weeks, regardless of biological matrix utilized (hair or fingernails). Although sensitivity for both hair (0.10–0.34) and fingernails (0.33–0.42) was low at all three EtG thresholds in detecting any alcohol use, the corresponding PPVs of 0.99–1.00 suggest that positive tests are, with almost 100% certainty, true positives. EtG in hair as a qualitative indicator of any alcohol use (threshold 45 pg/mg) is consistent with previous research [11] and, based on the
results of this study. EtG in fingernails may be an alternative when a hair sample cannot be obtained [8]. The results of this study also suggest that EtG may have potential as a quantitative indicator, especially in fingernails, to detect varying levels of risk drinking, a finding consistent with previous research [12]. Although EtG in hair at the 8 pg/mg threshold detected 93% of high-risk drinkers (specificity 0.71), EtG in fingernails at all three thresholds detected 100% of high-risk and 82–86% of increasing-risk drinkers. Although clearly better in hair, a negative EtG fingernail test detected 64–79% of drinkers not at risk. Given this range of specificity and the corresponding NPVs (0.96–1.00), plus the identification of alcohol use factors associated with an incorrectly classified positive EtG fingernail test for increasing-risk drinking (number of drinking days and number of heavy drinking days in the past 12 weeks), the potential of EtG in fingernails as a quantitative indicator of alcohol use may hold promise. Such potential is supported further in that the EtG fingernail test discriminated between different types of drinkers more accurately than hair. In addition, the AuROC curve exploratory thresholds for EtG level in fingernails were higher in combined sensitivity and specificity than EtG level in hair for each alcohol use criterion. The exploratory thresholds for EtG level in fingernails of 37 pg/mg for increasing-risk drinkers and 56 pg/mg for high-risk drinkers also suggest that sensitivity and specificity of the EtG fingernail test may be increased with a threshold higher than the a priori thresholds of 8 pg/mg, 20 pg/mg and 30 pg/mg.

The strengths of this study include both a probability sample and a sample population of college students [14]. A study limitation may be the operationalization of the alcohol use measure as a weekly average, given that various alcohol use definitions exist (for example, heavy drinking defined as 60 g of pure ethanol/day [12]). Our operationalization, however, was based in part on low- and high-risk US drinking definitions [18,24] and supported by correlational analyses with EtG concentration (0.53, CI = 0.46, 0.59 for hair; 0.57, CI = 0.50, 0.63 for fingernails).

Alcohol biomarkers should be viewed as data that can supplement self-report or other information gathered by trained professionals [2]. Given the potential harms resulting from biomarker misclassification [2], practitioners should understand the advantages and disadvantages of alcohol biomarker use in various settings [2,29]. If used as a treatment monitoring tool, patients and clinicians need to be informed regarding possible extraneous, ethanol-containing exposures [1,29]. Nevertheless, hair and fingernails are easy to collect and serve as a relatively acceptable, unobtrusive alcohol use monitoring strategy [3]. Within this context, our findings support EtG in hair and fingernails as an objective long-term, up to 12 weeks, qualitative indicator of any alcohol use [11]. Pending further calibration research, for example, the use of other alcohol use measures or the combination of such measures, considerable potential may exist for EtG, especially in fingernails, as a long-term quantitative indicator of risk drinking.

**Declaration of interests**

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**Table 3** Logistic regression analysis of ethyl glucuronide (EtG) in fingernails in increasing risk drinkers (≥15 standard drinks/week): incorrectly classified positives versus correctly classified negatives (n = 382).

| Incorrect classification (versus correct classification) | Incorrect classification (versus correct classification) | Incorrect classification (versus correct classification) |
|--------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| 30 pg/mg OR (95% CI) | 20 pg/mg OR (95% CI) | 8 pg/mg OR (95% CI) |
| Gender | Race/ethnicity | Number of drinking days | Number of heavy drinking days |
| Male | Minority | 1.01 (0.55, 1.86) | 0.96 (0.42, 2.16) | 1.03 (1.00, 1.06)* | 1.17 (1.11, 1.23)** |
| Female | Non-minority | Ref# | Ref | Ref | Ref |
| Female | Ref | 1.27 (0.72, 2.24) | 0.78 (0.36, 1.72) | 1.03 (1.00, 1.06)* | 1.17 (1.11, 1.23)** |
| Female | Ref | 1.24 (0.72, 2.14) | 0.96 (0.47, 1.95) | 1.03 (1.01, 1.06)* | 1.17 (1.11, 1.23)** |

#Reference category; *P < 0.05; **P < 0.001. CI = confidence interval; OR = odds ratio.
and Dr Plate are employed by United States Drug Testing Laboratories, Inc. (USDTL). USDTL is a commercial reference laboratory that analyzes biological specimens for the presence of alcohol and drug biomarkers. Mr Lewis has an ownership interest in USDTL. Drs Berger, Fendrich and Fuhrmann were funded for their work on this study by a subaward from USDTL to the University of Wisconsin-Milwaukee.

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