

## Ethyl glucuronide in hair and urine – examinations on actual cut-off values

**Maria Elena Albermann, Frank Musshoff, Burkhard Madea**

Universitätsklinikum Bonn, Institut für Rechtsmedizin, Forensische Toxikologie, Stiftsplatz 12, D-53111 Bonn

---

**Key words:** Ethyl glucuronide; Urine; Hair; Abstinence test; Cut-off levels

### Abstract

**Aim:** Urine and hair testing for ethyl glucuronide (EtG) is used to determine previous alcohol intake and is utilized to document alcohol abstinence. The current German cut-off values for abstinence tests are 7 pg/mg in hair and 0.1 mg/l in urine. To verify these values, a retrospective analysis of determined EtG concentrations in urine and hair was performed.

**Methods:** 835 hair samples and 150 urine samples originating from driving ability diagnostics were included in this study. All EtG concentrations were determined using LC-MS/MS.

**Results and Discussion:** Measured EtG concentrations were between 0 and 232 mg/l in urine and between 0 and 161 pg/mg in hair. In over half of both urine and hair samples, concentrations below the LOQ were measured. Concentrations near the cut-off values were determined in less than 10% of all urine samples and less than 5% of all hair samples. A slight decrease or increase has only minor effects on the number of positive/negative results for both cut-off values.

**Conclusion:** Most of the analyzed hair and urine sample were tested negative for EtG. Only considerably lower cut-off values would increase the number of positive results significantly. This would, however, increase the risk of false positive results due to unconscious consumption of alcohol. In our opinion, adapting these values is not necessary for the above mentioned reasons.

### 1. Introduction

Ethyl glucuronide (EtG) measured in urine or hair is a powerful marker for previous alcohol intake and abuse. Several analytical procedures for the quantification of EtG in hair and urine have been developed so far. In many laboratories a considerable number of EtG analyses are performed routinely. Current German cut-off values for testing abstinence in the context of driving ability diagnostics are 7 pg/mg in hair and 0.1 mg/l in urine [1,2].

Up to now, either decreasing or increasing the cut-off values is still discussed with the aim to avoid false negative as well as false positive results. To verify the current cut-off values, a retrospective analysis of measured EtG concentrations in hair and urine was performed.

### 2. Material and Methods

Measured EtG concentrations of 835 hair and 150 urine samples were included in this study. All samples originated from driving ability diagnostics. For this reason, there is no reliable data on drinking behavior. EtG concentrations in urine and hair were measured using validated LC-MS/MS procedures [3,4]. In case of urine, a protein participation was performed. Hairs were extracted with water. Chromatographic separation was performed on a Synergi Polar-RP column (Phenomenex), using formic acid (0.1%) and acetonitrile as mobile phases. The LOD and LOQ for urine and hair were 0.005 mg/l and 0.019 mg/l and 1 pg/mg and 4 pg/mg, respectively.

### 3. Results and Discussion

#### 3.1. Ethyl glucuronide in urine

150 urine samples with determined EtG concentrations between 0 and 232 mg/l were included in this study. In over half of all cases (59%) concentrations below the LOQ were measured. 83% of the samples were tested negative for EtG ( $c_{EtG} < 0.1$  mg/l). In 95% of these samples concentrations below 0.055 mg/l were measured. Only 25 urine samples showed a concentration above 0.1 mg/l. Figure 3 shows the distribution of EtG concentrations in urine.

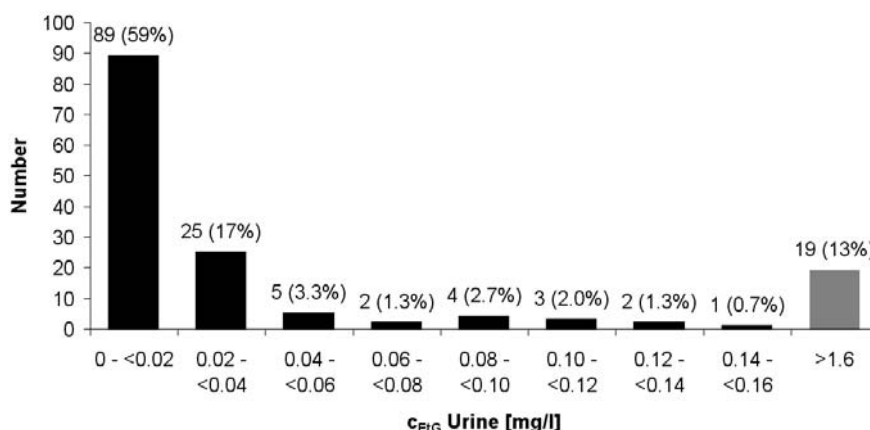


Fig. 1. Distribution of the determined EtG concentration in 150 urine samples (LOD = 0.005 mg/l and LOQ = 0.019 mg/l).

A variation of the cut-off value only leads to minor changes. Lowering the cut-off value to 0.05 mg/l would lead to 8 additional positives. Only 5 additional urine samples would be declared as negative using a higher cut-off value (0.15 mg/l).

Tab. 1. Consequences of a variation of the cut-off value in urine.

Cut-off value	Additional positives	Additional negatives
0.05 mg/l	8 (5.3%)	-
0.15 mg/l	-	5 (3.3%)

#### 3.2. Ethyl glucuronide in hair

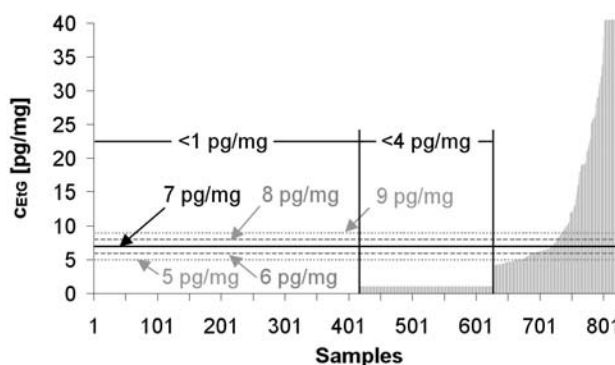


Fig. 2. Determined EtG concentration in 835 hair samples. Concentrations between LOQ (4 pg/mg) and LOD (1 pg/mg) are set to LOD, and those below are set to 0 mg/l.

The determined EtG concentrations of 835 hair samples were included in this analysis. Concentrations between 0 and 161 pg/mg were measured. In 50% of the hair samples no EtG was found ( $c_{\text{EtG}} < \text{LOD}$ ). EtG concentrations between the LOD and the LOQ were determined in 25% of the hair samples. In 95% of the negatively tested hair samples ( $c_{\text{EtG}} < 7 \text{ pg/mg}$ ) concentrations below 5.6 pg/mg were measured. All determined EtG concentrations are shown in figure 2. Figure 3 shows the distribution of EtG concentrations.

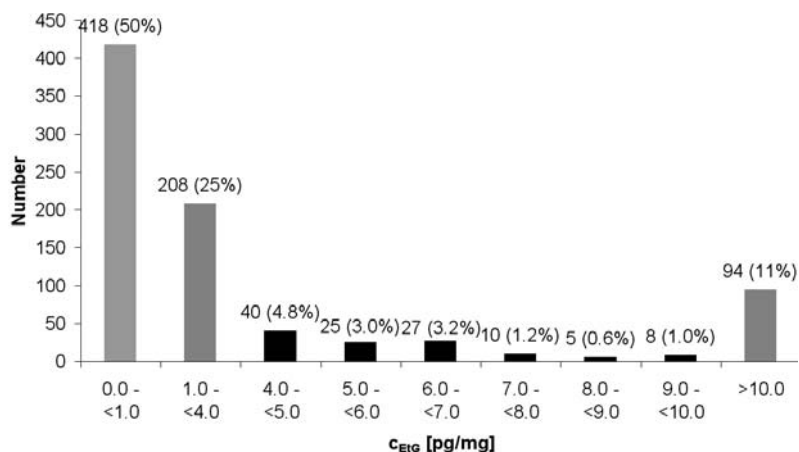


Fig. 3. Distribution of the determined EtG concentration in hair (LOD = 1 pg/mg and LOQ = 4 pg/mg).

A variation of the cut-off value in hair also leads only to minor changes. 27 (3.2%) additional positive hair samples are the result when theoretically lowering the value to 6 pg/mg. An increase of the cut-off value to 8 pg/mg decreases the number of positive hair samples by 1.2% (10).

Tab. 2. Consequences of a variation of the cut-off value in hair.

Cut-off value	Additional positives	Additional negatives
5 pg/mg	52 (6.2%)	-
6 pg/mg	27 (3.2%)	-
8 pg/mg	-	10 (1.2%)
9 pg/mg	-	15 (1.8%)

#### 4. Conclusion

As expected for abstinence tests, most of the analyzed hair and urine sample were tested negative for EtG. In many cases, EtG could not be detected at all. Positively tested hair and urine samples often show EtG concentrations far above the cut-off levels. Concentrations near the cut-off values were determined in less than 10% of all urine samples and less than 5% of all hair samples.

Kronstrand et al. recently found negative EtG results in some hair samples of persons who drank 16-32 g alcohol per day over the previous three months [5]. According to these results, a lower cut-off value would be essential to monitor strict abstinence. But considering the results of this study, only a considerably lower cut-off value would increase the number of positive results significantly. This involves a higher risk of false positive results due to unconscious consumption of alcohol.

Concentrations above 0.1 mg/l in urine can also be achieved for a very limited time period after uncommon use of alternative ethanol sources (foods, cosmetics/sanitizers) [6]. A higher

cut-off level in urine would achieve better sensitivity but also lead to a significant reduction of the detection window. In abstinence programs (with a 24-h waiting period) low-dose alcohol consumption would not be detected when using a higher cut-off level (e.g., 0.3 or 0.5 mg/l). To avoid such false positive results, all participants were enlightened concerning the risks of misleading results due to items that contain ethanol.

Lowering or increasing both cut-off values has only minor effects on the number of positive/negative results. In our opinion, adapting these values is not necessary for this reason.

## 5. References

- [1] Schubert W, Mattern R. Beurteilungskriterien zur Fahreignungsdiagnostik 2nd Edition Kirschbaum Verlag, Bonn Germany 2009.
- [2] Pragst F, Yegles M. Determination of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in hair: A promising way for retrospective detection of alcohol abuse during pregnancy? *Ther Drug Monit* (2008);30:255-263.
- [3] Albermann ME, Musshoff F, Madea B. A fully validated high performance liquid chromatography-tandem mass spectrometry method for the determination of ethyl glucuronide in hair for the proof of strict alcohol abstinence. *Anal Bioanal Chem* 2010;396:2435-2440.
- [4] Albermann ME, Musshoff F, Madea B. A high performance liquid chromatographic-tandem mass spectrometric method for the determination of ethyl glucuronide and ethyl sulfate in urine validated according to forensic guidelines. *J Chromatogr Sci* 2011;accepted.
- [5] Kronstrand R, Brinkhagen L, Nyström F H. Ethyl glucuronide in human hair after daily consumption of 16 or 32 g of ethanol for 3 month. *Forensic Sci Int.* 2011; doi:10.1016/j.forsciint.2011.01.44.
- [6] Musshoff F, Albermann E, Madea B. Ethyl glucuronide and ethyl sulfate in urine after consumption of various beverages and foods – misleading results? *Int J Legal Med* 2010;124:623-630.