Detection times of ethyl glucuronide in whole blood samples from heavy drinkers determined by a sensitive UPLC-MS/MS method

M. Böttcher[1], O. Rönitz[1], O. Beck[2], N. Dahmen[3], A. Helander[2]

[1]MVZ Labor Dessau GmbH, Dessau, Germany

[2]Karolinska Inst. and Karolinska Univ. Laboratory, Stockholm, Sweden

[3]Fachklinik Katzenelnbogen, Katzenelnbogen, Germany

Introduction

Ethylglucuronide (EtG) is a conjugated metabolite of ethanol which has gained widespread use as a biomarker to monitor alcohol abstinence in treatment programs and other settings. EtG is measured most often in urine samples with detection times up to several days after alcohol ingestion. However, there are several **disadvantages** associated with EtG testing in urine:

1. the sample often has to be taken under supervision
2. urinary EtG concentrations are influenced by diuresis
3. glucuronidases from bacterial contamination may cause false negative results
4. postcollection bacterial synthesis of EtG may cause "clinical" false positive results

These points would play no role if EtG testing could be performed in serum or whole blood (WB). However, compared with urine, less is known about the time course of EtG in serum or WB after alcohol intake and the clinical value of serum/WB testing.

The objective of this study was to evaluate WB as an alternative matrix to urine for EtG testing. A sensitive UPLC-MS/MS method for EtG was developed and the detection times of EtG in paired WB (**cutoff 1 ng/mL**) and urine (cutoff 100 ng/mL) samples from heavy drinkers were compared.

Methods

Patient samples: Randomly selected alcohol-dependent patients being hospitalized at Universitaetsklinikum Mainz (Germany) for alcohol detoxification participated in this study; see "Patient data" and Tab. 2. The study was approved by the ethics committee at the University of Mainz.

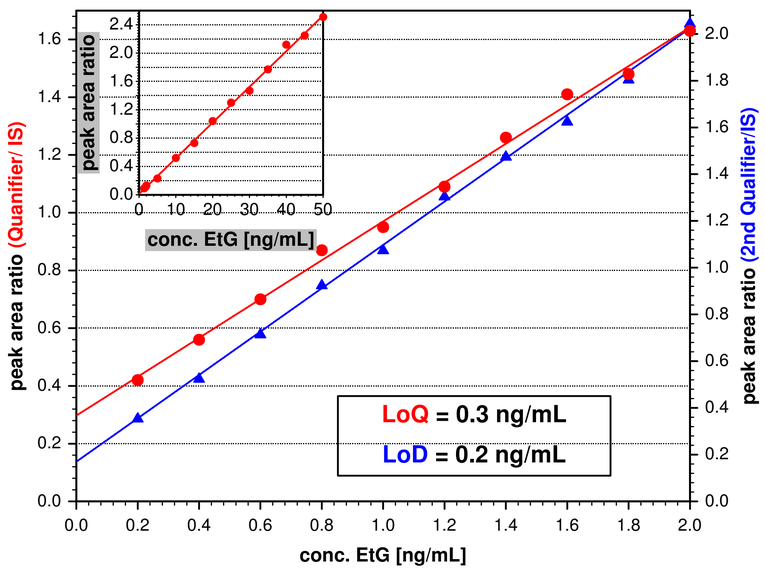
Measurement of EtG: Urine samples were analysed with our accreditated routine forensic method with a cutoff at 100 ng/mL. Serum or WB (10 µL) was fortified with 50 µL internal standards in MetOH (0.2 ng/mL EtG-d5, LGC Standards, Germany; 4 ng/mL EtG-d3, Carbosynth, UK). After centrifugation the supernatant was evaporated to dryness with N_2. The residue was dissolved in 50 µL 0.1% formic acid and frozen at -80°C. After thawing and centrifugation, 5 µL was injected into the UPLC-MS/MS (Waters Acquity UPLC connected to a Waters Xevo TQ-S). Separation was achieved within 4.5 min on a Waters 2.1x150 mm, 1.8 µm, HSS T3 column kept at 40°C by gradient elution from 2 to 100% MetOH with 0.1% formic acid at a flow rate of 0.3 mL/min. The system was operated in ESI-negative and SRM mode and 3 transitions were monitored: **EtG:** 221.0 > 85.0 (quantifier-ion), 221.0 > 75.0, 221.0 > 113.1 ; **EtG-d5:** 226.0 > 85.0 (quantifier-ion), 226.0 > 75.0, 226.0 > 113.1 ; **EtG-d3:** 224.1 > 85.0 (quantifier-ion), 224.1 > 75.0, 224.1 > 113.1. Serum calibration was performed from 0.2 to 2.0 ng/mL (n = 10) and from 1.4 to 50 ng/mL (n = 13), see Fig. 1. The cutoff for serum and WB was set at 1 ng/mL. Ion suppression for serum and WB was excluded by direct infusion of the analytes and injection of 10 different EtG negative samples each prepared as described above. A commercial serum control (ACQ Science, Germany) was differently diluted (1:32, 1:500, 1:900) with negative serum and used as QC sample (see Tab. 1).

Conclusion

-- Fig. 4: EtG positive rate was slightly better in WB than in urine at the selected cutoffs (WB = 1ng/mL, urine = 100 ng/mL) suggesting that WB could be of equal value.
-- Fig. 5+6: EtG detection time was comparable for urine (median: 98 h, 5%-95%: 30 h - 176 h) and WB (median: 111 h, 5% - 95%: 43 h - 168 h).
-- Fig. 5c+6c: In WB there is a much better correlation between alcohol dose (initial breath alcohol conc.) and EtG detection time than in urine (diuresis!).
-- WB or "semi-invasively" drawn capillary blood seems to be a possible alternative to urine in EtG testing.

UPLC-MS/MS method

Fig. 1 Serum calibration for EtG - working- and cutoff-range



cutoff-range (0.2 ng/mL - 2.0 ng/mL)	cutoff-range (0.2 ng/mL - 2.0 ng/mL)	working-range (1.4ng/mL - 50 ng/mL)
peak area ratio (Quantifier/ IS) linear regression: (n = 10) y = a + bx 0.2 <= x <= 2 a = 0.2973 b = 0.6724 r = 0.9984	peak area ratio (2nd Qualifier/IS) linear regression: (n = 10) y = a + bx 0.2 <= x <= 2 a = 0.17 b = 0.9273 r = 0.9993	peak area ratio (Quantifier/IS) linear regression: (n = 13) y = a + bx 1.4 <= x <= 50 a = 0.0112 b = 0.0504 r = 0.9991
IS = EtG-d5 (1 ng/mL)		IS = EtG-d3 (20 ng/mL)

Tab. 1 EtG serum controls

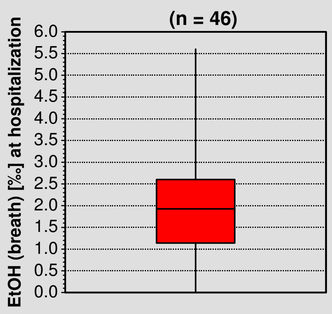
n	ACQ-Ctrl. EtG 3/10-A-SE	target conc. [ng/mL]	mean	CV [%]
62	1:20	32.0	32.9	10.7
48	1:500	1.28	1.23	17.4
49	1:900	0.71	0.73	16.6

Patient data

University hospital Mainz, heavy drinkers

n = 46; 36 male: 25 to 74 years, 10 female: 32 to 68 years; pats. hospitalized for alcohol detoxification; day of hospitalization (day 0): BrAC testing and interview.

paired samples:
urine spls. from day 1 (0.83 h - 48.25 h) to day 7. WB spls. from day 1 (0.17 h - 47.8 h) to day 5 and at day 7.
Serum spls. only at day 1



Tab. 2 Patient samples collected

patient	Urine WB	patient	Urine WB	patient	Urine WB	patient	Urine WB	patient	Urine WB	patient	Urine WB
1	1-7	10	1-6	19	1-7	28	1-7	37	1-7	46	1-7
	1-7		1-5		1-5		1-7		1-7		1-7
2	1-5	11	1-7	20	1-7	29	1-7	38	1-7		
	1-5		1-7		1-7		1-7		1-7		
3	1-7	12	1-7	21	1-7	30	1-7	39	1-7		
	1-7		1-7		1-7		1-7		1-7		
4	1-7	13	2-6	22	1-7	31	1-7	40	1-7		
	1-7		1-7		1-7		1-7		1-7		
5	1-7	14	1-7	23	1-7	32	1-7	41	1-7		
	1-7		1-7		1-7		1-7		1-7		
6	1-7	15	1-6	24	1-7	33	1-7	42	1-7		
	1-7		1-5		1-7		1-7		1-7		
7	1-7	16	1-7	25	1-7	34	1-7	43	1-7		
	1-7		1-7		1-7		1-7		1-7		
8	1-7	17	1-7	26	1-7	35	1-7	44	1-7		
	1-7		1-7		1-7		1-7		1-7		
9	1-7	18	1-7	27	1-7	36	1-7	45	1-7		
	1-7		1-7		1-7		1-4+6-7		1-7		

Results

Fig. 2 Correlation of EtG serum and urine conc. at day 1

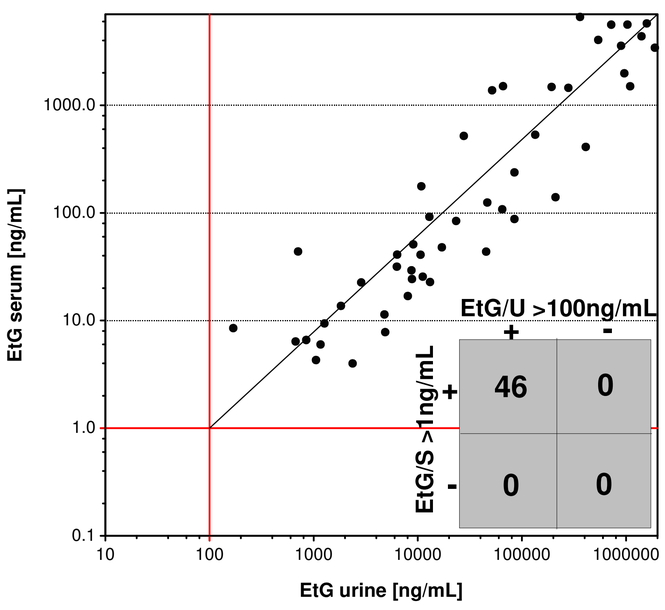


Fig. 3 (Dis-) Agreement of EtG WB and serum conc. at day 1

Fig. 3a Low concentration range

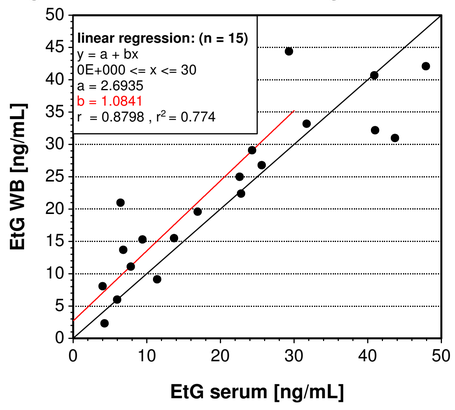


Fig. 3b High concentration range

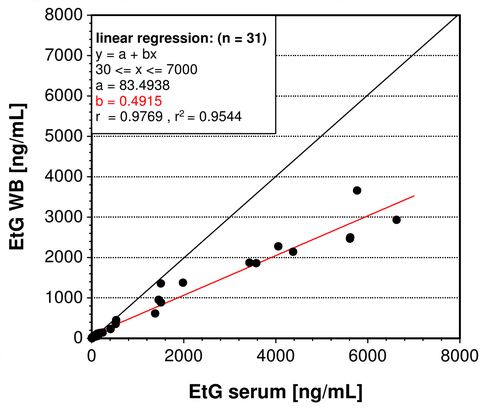


Fig. 4a Correlation of EtG WB conc. vs. urine conc., day 1 to day 7

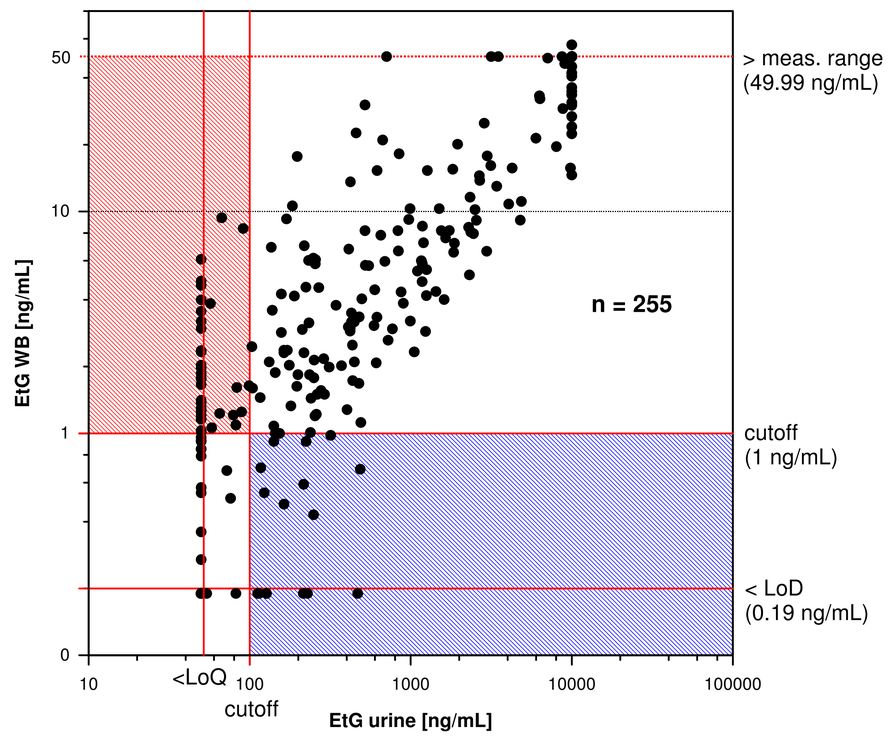


Fig. 4b Qualitative evaluation

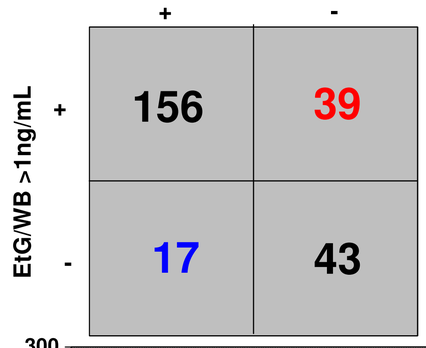


Fig. 4c Influence of diuresis

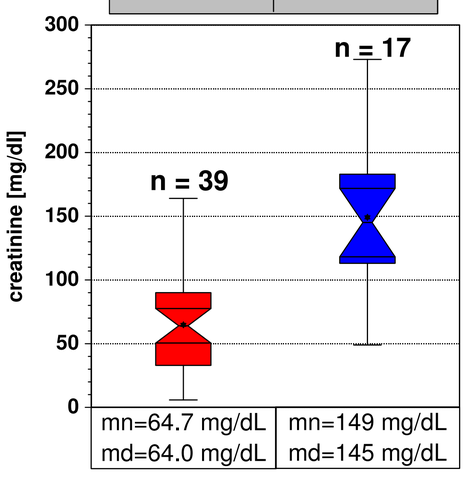


Fig. 5a EtG elimination in urine

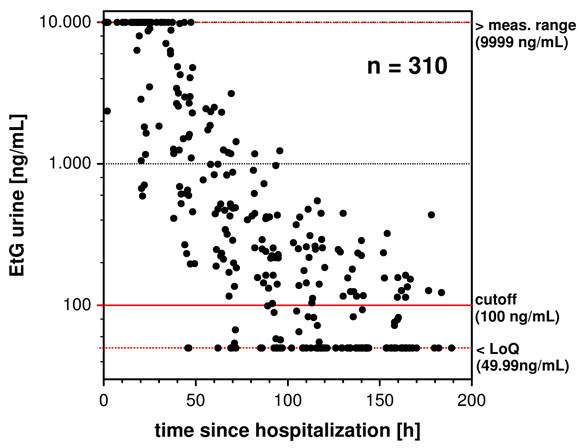


Fig. 5b Urine: detection times cutoff 100 ng/mL

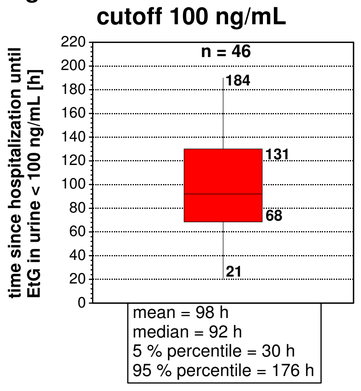


Fig. 5c Urine: detection time and dose

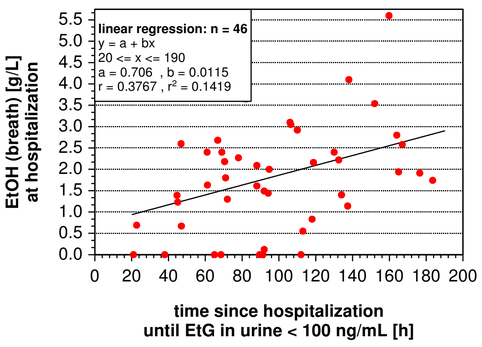


Fig. 6a EtG elimination in WB

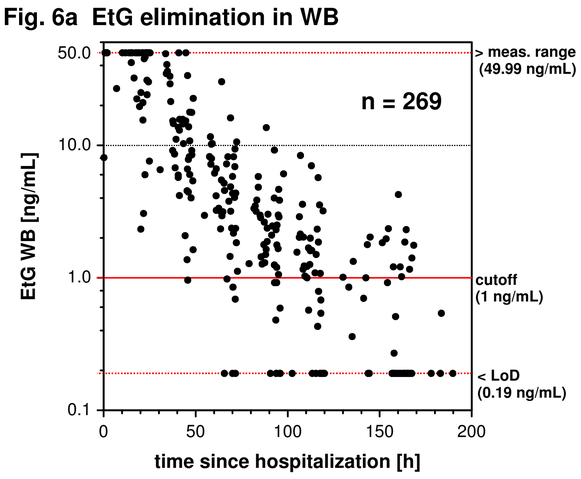


Fig. 6b WB: detection times cutoff 1 ng/mL

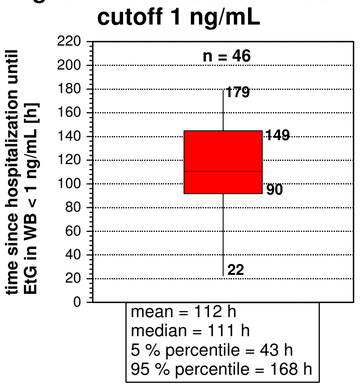


Fig. 6c WB: detection time and dose