False positive Ethyl Glucuronide resulting from incidental alcohol exposure

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INTRODUCTION

Ethyl Glucuronide (EtG) is a water soluble, stable, non-volatile, direct metabolite of ethanol. Metabolism occurs as soon as alcohol enters the blood stream. More than 90% of alcohol consumed is metabolised in the liver, via the action of the enzyme alcohol dehydrogenase (ADH). ADH catalyses the reaction in which alcohol is oxidised to acetaldehyde. This is then metabolised to acetate by aldehyde dehydrogenase and then to carbon dioxide. Other enzymes capable of metabolising alcohol include; catalase, the cytochrome P450 enzyme, CYP2E1 and microsomal oxidising systems (MEOS). Between 5-8% of unchanged alcohol is excreted in the urine, sweat and breath. The elimination of ethanol by enzymatic conjugation with glucuronic acid represents approximately 0.5 to 1.5% of the total ethanol elimination.

Whilst the detection period for alcohol is relatively short, EtG can be detected for up to 80 hours and peaks at approximately 4 hours after alcohol consumption. EtG offers several advantages over traditional markers of alcohol abuse such as gamma glutamyl transferase (GGT), mean corpuscular volume (MCV) and carbohydrate deficient transferrin (CDT). It is a direct, specific and sensitive marker of alcohol consumption, being present only if ethanol is consumed. It is not influenced by age, gender, medication or non-alcohol related diseases and is not dependant on chronic alcohol consumption. EtG does not accumulate during chronic alcohol intake.

For these reasons EtG is proving a promising biomarker for monitoring alcohol abstinence, where zero tolerance policies are in existence. Such examples include liver transplant recipients, recovering alcoholics in withdrawal treatment programmes and some fields of employment such as aviation or other transportation. However, ethanol is not only contained in alcoholic beverages. It can be found in a wide range of every day products including; foods, medicines, mouthwashes, perfumes, hygiene products, disinfectants, hand sanitisers and automotive fuel. The alcohol in these products may enter the body via oral consumption, absorption or inhalation. We investigated the effect of incidental alcohol consumption, from mouthwash, on urinary EtG concentrations.

Figure 1. A selection of every day products containing alcohol (a) and a close up of the Buttercup cough, cold and sore throat syrup packaging detailing the equivocal alcohol dosage (b).

EXPERIMENTAL

Five commonly available, alcohol containing mouth washes (table 1) were purchased and used as directed. Five female volunteers (<30 years old), abstained from alcohol for a minimum of 3 days. Urine samples were then obtained immediately before (control) and 4 hours (+/- 15 minutes) after use of the mouth washes. These were analysed for EtG using the Microgenics DRI® EtG Enzyme Immunoassay on the Olympus AU400 platform. Assays were semi-quantitative (0, 100 (LLOQ), 500, 1000, 2000 (ULOQ) ng/mL) with 4 QC levels employed (375, 625, 750, 1250ng/mL). The sensitivity of the assay is quoted as being 15.3ng/mL, but cut offs of 500ng/mL or 1000ng/mL are typically recommended for monitoring alcohol abstinence in alcohol rehabilitation programs. Unlike urinary excretion of ethanol, EtG concentrations are highly influenced by water intake. Normalisation of EtG to creatinine is recommended. Creatinine, measured using the Jaffe reaction on the Siemens Advia 2400 and ethanol concentrations measured by head space GC-FID on a Shimadzu GC 2014 coupled to a HTA, HT200H head space auto sampler, were used for comparison.

Brand	Manufacturer	Flavour	Dose	Duration	Alcohol %
Plax Multi-Protection	Colgate	Cool mint	20mL	30 sec	6.5%
Smile Totalcare antiseptic	Boots	Fresh mint	Half cap	Up to 60 sec	12.1%
Macleans	GalaxoSmithKline	Fresh mint	15mL	60 sec	15.0%
Listerine antibacterial	Pfizer	Fresh mint	20mL	30 sec	22.7%
Corsodyl	GalaxoSmithKline	Mint	10mL	60 sec	7.0%

Table 1. The mouth washes tested and their % alcohol content.

RESULTS & DISCUSSION

All test and control samples were negative for ethanol (<10mg/dL). Four of the five subjects were negative for EtG after use of all mouth washes tested. One subject yielded elevated results (84-180ng/mL) for the mouth washes compared to the other subjects (<15.3-59ng/mL). Previous tests have also shown this individual to give higher urinary EtG concentrations than other subjects tested. These findings may result from incidental alcohol consumption via another route or possibly EtG synthesis in infected urine. It is possible that inter-individual variations in EtG production exist. Whatever the source, it is apparent that EtG positives may result from incidental alcohol exposure in individuals who knowingly abstain from alcoholic beverage consumption. Until more is known about the effects every day alcohol containing products have on EtG production, care should be taken in applying cut offs and interpreting these results.

Subject		1		2		3		4		5	
Mouth wash		EtG	Creatinine	EtG	Creatinine	EtG	Creatinine	EtG	Creatinine	EtG	Creatinine
		ng/mL	mmol/L	ng/mL	mmol/L	ng/mL	mmol/L	ng/mL	mmol/L	ng/mL	mmol/L
Plax	Before	ND	11.5	ND	5.0	ND	4.3	ND	4.7	ND	2.0
	After	ND	6.1	ND	2.9	ND	2.7	ND	4.1	ND	3.5
Boots Smile	Before	ND	17.6	ND	7.3	ND	4.5	ND	6.4	139	3.4
	After	ND	6.2	ND	2.7	ND	5.8	ND	6.3	147	10.7
Macleans	Before	ND	14.8	ND	6.6	ND	2.7	ND	13.4	118	2.1
	After	ND	7.7	ND	8.5	ND	4.5	ND	3.2	180	5.2
Listerine	Before	ND	7.0	ND	10.1	ND	10.0	ND	2.8	ND	1.7
	After	ND	4.7	ND	12.3	ND	3.5	ND	2.9	ND	3.6
Corsodyl	Before	ND	6.5	ND	11.3	ND	1.9	ND	9.9	100	2.8
	After	ND	11.0	ND	1.6	ND	13.6	ND	2.5	ND	3.0

ND = None detected (<100ng/mL)

Table 2. EtG and Creatinine results following use of 5 brand name mouth washes.

False positives (50 to >300ng/mL) resulting from the use of mouth wash have been reported previously (Constantino *et al*). However, volunteers gargled with the mouth wash for 15 minutes or three times daily, which is unlikely to be representative of typical usage. This study has investigated the effects of mouth wash on EtG concentrations following one off doses in accordance with the manufacturers instructions. It is possible that mouth ulcers or increased blood circulation to the gums, stimulated by brushing before use, could result in further increases in EtG concentration.

CONCLUSION

EtG monitoring is complimentary to clinical assessment in alcohol withdrawal programmes and provides an early warning of lapses or relapses. Previous to this, determined individuals could drink at times when testing was unlikely, due to the rapid excretion of alcohol. However, further work is required to establish the effect of false positives resulting from incidental alcohol exposure, such as from medication, hygiene products, cosmetics and foods, and the cut off concentration reviewed accordingly. This is of particular importance in countries such as Germany, where 1 year alcohol abstinence is required for drivers who have lost their licences following a charge of driving impairment, where liver transplantation is conditional on abstinence and where the loss of employment, child custody or other privileges may result.

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