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False-positive ethyl glucuronide immunoassay screening caused by a propyl alcohol-based hand sanitizer

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ABSTRACT

Background: Urine ethyl glucuronide (EtG) is considered as a specific marker of recent ethanol consumption. We describe false-positive DRI® EIA EtG enzyme immunoassay results caused by propyl glucuronides in urine after using a propanol-based hand sanitizer.
Methods: EtG screening was done with the DRI® EIA EtG assay (Microgenics), using a cut-off of 0.5 mg/L as recommended by the manufacturer and of 0.1 mg/L as demanded by the German Regulations for Reissuing Drivers Licenses. Confirmatory EtG analysis was done with the ClinMass® EtG LC–MS/MS testkit (Recipe), extended by the mass transitions 235.1 → 75.1, 235.1 → 85.1, and 235.1 → 113.1 for the detection of the 1- and 2-propyl glucuronides. Self-experiments were done by staff members of our lab (n = 7), using 3 mL Sterillium® Classic Pure (30 g/100 g 1-propanol and 45 g/100 g 2-propanol) for hand sanitation every quarter of an hour for 8 h according to DIN EN 1500:2011-05 with and without an exhauster and by passive inhalation of the sanitizer vapor. Spot urine samples were taken immediately before and up to 24 h after the first sanitizer use.
Results: False-positive immunoassay results of up to 4 mg/L or 2.3 mg/g creatinine were obtained after normal use of the sanitizer and also after passive inhalation of the sanitizer vapor (up to 0.89 mg/L or 0.61 mg/g). Immunoassay results were positive even after 4-fold use of the sanitizer (up to 0.14 mg/L or 0.38 mg/g) and up to 6 h after the last sanitizer contact (maximum 0.63 mg/L and 0.33 mg/g for sanitizer users and 0.25 mg/g after passive inhalation). Spiking of EtG-free urine with 1-propyl glucuronide (Athena Environmental Sciences) between 0.05 and 10 mg/L clearly demonstrated a cross reaction of the immunoassay of approx. 10% as compared to EtG. LC–MS/MS of urines with a positive immunoassay EtG result did not show EtG signals, but distinct signals of 1-propyl glucuronide (n-propyl glucuronide) and 2-propyl glucuronide (iso-propyl glucuronide). An exhauster effectively prevented the inhalation of the sanitizer vapor, the formation of propyl glucuronides and thus false-positive DRI® EIA EtG screening results, proving that propyl alcohols are almost exclusively taken up by respiration.
Conclusions: The widespread use of propanol-containing products such as hand sanitizers may lead to sufficient uptake of propyl alcohols and excretion of significant amounts of propyl glucuronides to yield false-positive DRI® EIA EtG screening results. Thus, positive EtG immunoassay results have to be controlled by mass-spectrometry, in clinical cases at least if ethanol intake is denied by the patient.

1. Introduction

Ethyl glucuronide (EtG), a minor hepatic metabolite of ethanol, is increasingly used as a marker of recent ethanol consumption (Review in [1]). Current EtG analysis techniques allow trace analysis of EtG, e.g. detection limits of 0.005 mg/L in urine [2] and thus can demonstrate the intake of as little as 1 g of ethanol [3]. As a consequence, there is an increasing number of reports on true-positive EtG results despite alleged abstinence, e.g. by (hidden) ethanol in foods, ethanol-based sanitizers, cosmetics etc. or even by an EtG containing hair care product [4–9]. Additionally, false-positive immunological EtG screening results, caused by trichloroethyl glucuronide, the hepatic metabolite of the sedative chloralhydrate, have been reported [10].

In our lab urine samples from drug addicts on methadone- or buprenorphine-treatment frequently yield positive DRI® EIA EtG screening results at 0.1–0.3 mg/L, exceeding the recommended cut-off level [11], yet unconfirmed by LC–MS/MS. Questioning of such patients in some cases revealed extensive use of iso-propanol

Abbreviation: EtG, ethyl glucuronide.

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(2-propanol) in household cleaners or sanitizers. Since such products are widely used, not only in private households but also in professional health care institutions like our lab, we became interested (a) whether a controlled use of propanol-based sanitizers according to DIN EN 1500:2011-05 standard "Chemical disinfectants and antiseptics – Hygienic handrub – Test method and requirements" [12] can cause false-positive DRI® EIA EtG results in urine, (b) whether this is due to propyl alcohol glucuronides (1-propyl glucuronide and/or 2-propyl glucuronide) formed after presumed transdermal or inhalative uptake of propyl alcohols and (c) how long these compounds are excreted after a common working day of 8 h with dozens of hand disinfections.

2. Materials and methods

Sterillium® Classic Pure containing (among others) 30 g/100 g 1-propanol (syn. n-propanol, propan-1-ol) and 45 g/100 g 2-propanol (syn. iso-propanol, propan-2-ol) [13] was purchased from Bode Chemie (Hamburg, Germany).

The DRI® EIA Ethyl Glucuronide test kit came from Microgenics (Passau, Germany) and the ClinMass® Ethyl Glucuronide and Ethyl Sulfate test kit from Recipe (Munich, Germany). EtG and EtS for validation purposes were from Lipomed (Bad Säckingen, Germany) and 1-propyl β-D-glucuronide (syn. 1-propyl glucuronide, n-propyl glucuronide) from Athena Environmental Sciences Inc. (Baltimore, USA). 2-propyl β-D-glucuronide (syn. 2-propyl glucuronide, iso-propyl glucuronide) is not commercially available. The retention time of the latter was established by analyzing urine samples after hand sanitation with 60% (v/v) 2-propanol. Structures of the ethyl and propyl glucuronides are shown in Fig. 1.

In our lab, hand disinfection is regularly done with Sterillium® Classic Pure. To investigate possible false-positive DRI® EIA EtG results and their time course [items (a) and (c) from introduction], five co-workers of our laboratory (2 female, 3 male) used the sanitizer every quarter of an hour for up to 8 h according to the DIN EN 1500:2011-05 standard [12] for hand disinfection. This was done in a room of approx. 5 m × 2.3 m × 3 m. Two other persons (1 female, 1 male) were also present in this room but did not use any sanitizer, serving as controls for passive inhalation.

To assess whether propyl alcohols from Sterillium® Classic Pure are absorbed by the skin or the respiratory tract [item (b) from introduction], the same procedure was followed by the same subjects on a different day, using an exhauster to avoid vapor inhalation.

Spot urine samples were collected into Urine Z Sarstedt Monovettes (Numbrecht, Germany) at baseline and 1, 2, 4, 6, 8, 10, 12, 14, and 24 h after the initial disinfection. Immunological EtG screenings were done in 2 analysis series, one after the 8 h sampling, the other after the 24 h sampling. Urines were stored at 4–8 °C for a maximum of 16 h until immunoassay analysis and for a maximum of 1 week until LC–MS/MS analysis.

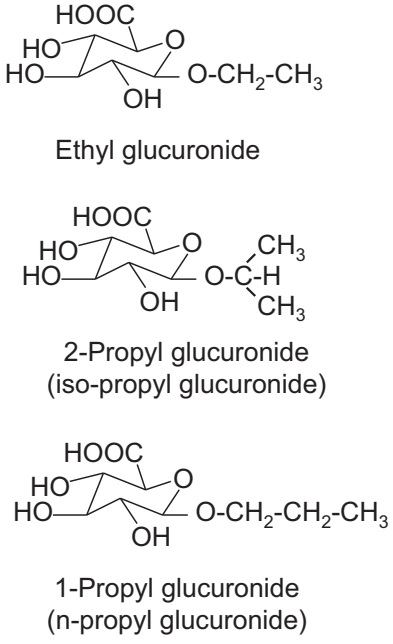

Fig. 1. Structures of ethyl glucuronide, 1-propyl glucuronide, and 2-propyl glucuronide.

Ethyl glucuronide screening was done with the DRI® EIA EtG enzyme immunoassay on a Beckmann Coulter AU 680 analyzer (Krefeld, Germany). This assay is a competitive immunoassay with ethyl glucuronide antibodies, glucose-6-phosphate dehydrogenase labeled ethyl glucuronide, and a final photometric detection step. A detailed evaluation of this immunoassay is given in [14]. In our lab, EtG screening results are regularly reported as concentrations, using a cut-off of 0.5 mg/L in accordance with the test instructions (package insert of the DRI® EIA EtG testkit), and additionally as EtG/creatinine ratios (cut-off 0.5 mg/g) to account for varying urine dilution [15]. An additional cut-off of 0.1 mg/L was implemented according to the German Guidelines for Reissuing Drivers Licenses [11]. In our routine lab, confirmatory analysis is done by LC–MS/MS if it is consented by the clinician.

Ethyl glucuronide and propyl glucuronides confirmatory analysis was done by LC–MS/MS, using the ClinMass® Ethyl Glucuronide testkit (Recipe, Munich, Germany). The samples were measured with a 1260 Infinity LC (Agilent, Waldbronn, Germany) coupled to a MistraSwitch column oven (Maylab Analytical Instruments, Vienna, Austria) and a QTRAP 5500 mass spectrometer (AB Sciex, Darmstadt, Germany); a PAL HTC-xt autosampler (CTC Analytics, Zwingen, Switzerland) was used for injections. Detailed analysis parameters are described in the test instructions (Field Manual Clin Mass® LC–MS/MS Complete Kit Ethylglucuronide and Ethylsulfate in Urine, Recipe MS8000, Version 1.1, 20.06.2011). For detection of the propyl glucuronides, the mass transitions m/z 235.1 → 75.1, m/z 235.1 → 85.1 and m/z 235.1 → 113.1 were added to the component table of the mass spectrometer. These mass transitions were established by direct infusion of a 1-propyl glucuronide solution into the mass spectrometer. Using this slightly modified Recipe LC–MS/MS application, we obtained 2 almost baseline separated signals in the ion chromatograms (Fig. 2), representing 1-propyl glucuronide (tested by spiking of pure 1-propyl glucuronide standard substance) and presumably 2-propyl glucuronide, which was unavailable as a reference compound. The retention time of this glucuronide was established by analyzing urine samples after hand disinfection with 60% 2-propanol (v/v).

Creatinine was determined on a Beckman Coulter AU 680 analyzer, using a modification of the Jaffe method (test kit from Thermo Fisher Scientific Microgenics, Passau, Germany).

3. Results

Multiple hand disinfection with 1- and 2-propanol-based Sterillium® Classic Pure lead to false-positive DRI® EIA EtG results of up to 4 mg/L or 2.3 mg/g creatinine in 6 of 7 subjects irrespective of whether the sanitizer was used actively or just inhaled (Fig. 3). One male neither had false-positive DRI® EIA EtG results (Fig. 3) nor propyl glucuronides after active use of the sanitizer in his urine as revealed by LC–MS/MS.

Four of 5 subjects who underwent active hand sanitation had positive DRI® EIA EtG results of up to 0.63 mg/L or up to 0.33 mg/g for more than 6 h after the last disinfection. The results of the 2 subjects exposed to mere inhalation of the sanitizer vapor exceeded 0.1 mg/L in both and increased to 0.89 mg/L and 0.61 mg/g creatinine in one subject who had still 0.25 mg/g creatinine 6 h after the last sanitizer exposition.

Each urine tested positive by the immunoassay showed distinct signals of 1- and 2-propyl glucuronide, but no signals for EtG in LC–MS/MS (Fig. 2). Additionally, spiking of EtG- and propyl glucuronide-free urine samples with pure 1-propyl glucuronide (0.05–10 mg/L) resulted in a positive EtG immunoassay result. The mean response factor of the immunoassay for 1-propyl glucuronide as compared to EtG was approx. 10% (Fig. 4). This proves the positive DRI® EIA EtG screening results to be due to cross reaction with propyl glucuronides.

Hand disinfection under an exhauster completely prevented false-positive DRI® EIA EtG screening results (Fig. 3) as well as the appearance of propyl glucuronides in urine.

4. Discussion

Natural sources of propyl alcohols (1-propanol and 2-propanol) are plants and propyl alcohol biosynthesis by microorganisms, e.g. during decomposition of organic material. Propyl alcohols are used e.g. as multipurpose solvents and intermediates in industrial chemistry or as odorants and flavors in food, as solvents and