EVALUATION OF A NEW IMMUNOASSAY FOR URINARY ETHYL GLUCURONIDE TESTING

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Abstract — Aims: The minor ethanol metabolite ethyl glucuronide (EtG) is used as a sensitive and specific test for recent alcohol consumption with clinical and forensic applications. This study evaluated a new enzyme immunoassay (DRI-EtG EIA, Microgenics Corp.) for determination of the EtG concentration in urine samples. Methods: Evaluation was done using the kit calibrators (range 0–5.0 mg/L) and controls, an external quality control sample, and 400 consecutive urines from the routine samples pool. The measuring range was extended by dilution of urine samples with saline. Comparison was made with an established liquid chromatographic-mass spectrometric (LC-MS) method. Results: The intra- and inter-assay imprecision of the DRI-EtG EIA in the range 0.4–2.5 mg/L was <2% (coefficient of variation, CV), and the limit of quantification was <0.1 mg/L. For the 400 urine samples, the EtG concentrations obtained using the DRI-EtG EIA (mean 24.2 mg/L, range 0–830) and LC-MS method (mean 22.4 mg/L, range 0–959) showed an overall good and statistically significant agreement (r² = 0.931, P < 0.0001). Conclusions: These results indicated a level of accuracy and selectivity of the DRI-EtG EIA for quantification of urinary EtG. In the absence of a commonly accepted cut-off limit for urinary EtG, a threshold of 0.5 mg/L (2.2 μmol/L) is proposed, to obtain a high sensitivity but avoid positive results due to unintentional ethanol exposure.

INTRODUCTION

Ethyl glucuronide (EtG) is a direct phase-II metabolite of ethanol formed by action of UDP-glucuronosyltransferase (UGT) (Foti and Fisher, 2005). EtG has long been known as a chemical entity but its undisputable occurrence as ethanol metabolite in humans was associated with the development of sensitive and specific mass spectrometric methods (Schmitt et al., 1995). EtG and ethyl sulphate (EtS), another recently discovered human phase-II ethanol metabolite (Helander and Beck, 2004), are minor metabolites each making up less than 0.05% of the ingested ethanol dose (Dahl et al., 2002; Helander and Beck, 2005), but they show much longer detection times than the parent compound (Borucki et al., 2005; Hoiseth et al., 2007). After the ethanol has been eliminated from the body, EtG and EtS remain measurable in the urine for another ~1–4 days in a dose-dependent manner (Schmitt et al., 1997; Dahl et al., 2002; Wurst et al., 2002; Borucki et al., 2005). The resulting high sensitivity for recent alcohol consumption is the basis for their increasing popularity as alcohol biomarkers in clinical and forensic applications (Bergström et al., 2003; Wurst et al., 2003; Jones, 2006; Hoiseth et al., 2007)

The development of accurate liquid chromatographic-mass spectrometric (LC-MS) methods for qualitative and quantitative determination of EtG (Stephanson et al., 2002; Weinmann et al., 2004; Bicker et al., 2006) allowed for introduction of urinary EtG as a routine test for detection of recent alcohol intake and for monitoring of abstinence. However, LC-MS methods are costly and only available in specialized laboratories, which have hindered a widespread clinical use of this test. A preliminary immunochemical screening method (ELISA) based on polyclonal antibodies was found not to be sensitive and specific enough (Zimmer et al., 2002), but another more promising prototype enzyme immunoassay (EIA) based on a monoclonal antibody was recently presented (Biń et al., 2006).

This study evaluated a new and potentially commercially available version of the EIA method, based on a new monoclonal antibody (DRI Ethyl Glucuronide Enzyme Immunoassay [DRI-EtG EIA]; Microgenics Corp.), for analysis of EtG in urine samples. Comparison was made with an established LC-MS method (Stephanson et al., 2002).

MATERIALS AND METHODS

The urine specimens used for the method comparison were surplus volumes of 400 consecutive de-identified samples from the routine pool at the Alcohol Laboratory, Karolinska University Hospital, Stockholm, for which the EtG concentration had been determined by an electrospray LC-MS method with selected ion monitoring (Stephanson et al., 2002). The lower limit of quantification (LOQ) of the LC-MS method is ~0.1 mg/L (limit of detection ~0.05 mg/L) but for routine clinical purposes a cut-off limit of 0.5 mg/L is applied. The samples were stored frozen at ~20°C where the EtG concentration is stable for long time (Stephanson et al., 2002).

The urine samples were shipped frozen to the Arztpraxis für Medizinische Mikrobiologie und Labordiagnostik in Dessau, Germany for evaluation of the DRI-EtG EIA on an Olympus AU640 instrument. The calibrators (range 0–5.0 mg/L [0–22.5 μmol/L] EtG in urine matrix), controls (0.38–1.25 mg/L in urine matrix), and instrumental application provided by the manufacturer were used together with an external quality control urine sample (EtG target range 1.90–3.50 mg/L) obtained from Medichem (Steinenbrom, Germany). The measuring range of the immunoassay was extended by dilution of urine samples with saline.
Owing to a known limitation of the Olympus instrument, urine samples with an optical density >2.5 at the 340 nm measuring wavelength of the DRI-EtG assay (N = 56; 14%) had to be diluted with saline (range 2–10 times, but typically 2 or 4 times) prior to analysis.

RESULTS AND DISCUSSION

The intra-assay imprecision, expressed as the coefficient of variation (CV; N = 10), for the kit controls was <1.7% and the bias from target values ranged from −8.1 to 8.0% (Table 1). The inter-assay CV (30 measurements on 5 separate days) was <2.2% with a mean bias ranging from −7 to 9.3%. The assay imprecision data demonstrated that the LOQ of the DRI-EtG EIA was <0.1 mg/L, and the test was able to detect 0.1 mg/L with good reproducibility. The intra- and inter-assay CV for the external EtG quality control sample was <1.8% with a bias of <9% from the target (Table 1).

The EtG concentrations obtained using the DRI-EtG EIA (mean 24.2 mg/L, range 0–830) and LC-MS (mean 22.4 mg/L, range 0–959) methods showed an overall good and statistically significant agreement ($r^2 = 0.931, P < 0.0001, N = 400$). For 126 urine samples with EtG values ranging from 0.10 to 50 mg/L by LC-MS method (i.e. using a maximum ten-fold dilution to get into the calibration range of the immunoassay), the $r^2$ was 0.961 ($P < 0.0001$), the intercept close to the origin and the slope close to 1, and only few outliers were identified (Fig. 1). The good overall quantitative agreement of the DRI-EtG EIA with the LC-MS results indicated a low cross-reactivity of the EtG antibody to other urinary constituents.

Two different cut-off limits for urinary EtG (0.5 and 1.0 mg/L, corresponding to 2.2 and 4.5 μmol/L) were evaluated for use with the DRI-EtG EIA. The agreement of qualitative results with the LC-MS method (reference) was 98.2% at both cut-offs (Fig. 2). At the 0.5 mg/L limit, the number of classified positive samples was 8% higher than at the 1.0 mg/L limit. Most of the differing results were due to a combination of assay imprecision and a concentration close to the applied threshold (Fig. 2). However, out of the 400 samples 5 urine samples (1.2%) with clearly deviating EtG results were identified. Two of these contained EtG levels between 0.5 and 1.0 mg/L according to the DRI-EtG EIA, while EtG was not detected by LC-MS method (<0.05 mg/L). The remaining three samples contained 3.7–9.6 mg/L EtG according to the EIA but concentrations below the clinical cut-off (<0.5 mg/L) by LC-MS method. All five samples that were positive for EtG by the EIA, but showed negative results or values below the clinical cut-off by LC-MS method, were positive for EtS although showing an unusually low EtG/EtS ratio (Dahl et al., 2002; Helander and Beck, 2005). The reason for this is unknown but the DRI-EtG EIA did not show a measurable cross-reactivity with EtS (data not shown).

This evaluation demonstrated that the new DRI-EtG EIA is a sensitive and specific method for quantification of EtG in urine samples, and it also offers a low and clinically

Table 1. Intra- and inter-assay imprecision data for the new DRI Ethyl Glucuronide Enzyme Immunoassay on an Olympus AU640 instrument

<table>
<thead>
<tr>
<th>Assigned EtG concentration (mg/L)</th>
<th>Specimena</th>
<th>Intra-assay imprecisionb</th>
<th>Inter-assay imprecisionb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean EtG (mg/L)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>0.10</td>
<td>Kit calibrator</td>
<td>0.10</td>
<td>5.4</td>
</tr>
<tr>
<td>0.38</td>
<td>Kit control</td>
<td>0.38</td>
<td>1.3</td>
</tr>
<tr>
<td>0.62</td>
<td>Kit control</td>
<td>0.61</td>
<td>1.2</td>
</tr>
<tr>
<td>0.75</td>
<td>Kit control</td>
<td>0.81</td>
<td>0.9</td>
</tr>
<tr>
<td>1.25</td>
<td>Kit control</td>
<td>1.28</td>
<td>0.9</td>
</tr>
<tr>
<td>2.70</td>
<td>External quality control</td>
<td>2.48</td>
<td>1.6</td>
</tr>
</tbody>
</table>

a All specimens used for the imprecision study were in urine matrix.

b Results are for ten measurements within the same run.

Results are for six measurements per day on 5 separate days (N = 30).
relevant measuring range (Stephanson et al., 2002). The upper measuring range can be extended by dilution of samples with saline, although this may not always be necessary from a clinical point of view. The low frequency of ‘false’ positive results observed by the DRI-EtG EIA is not considered alarming, as a positive test result from an immunochromatographic drug test (i.e. screening) should be regarded as preliminary and generally undergo confirmation by an alternative method (e.g. LC-MS/MS) before being reported. In the absence of a commonly accepted cut-off limit for urinary EtG, a threshold of 0.5 mg/L (2.2 μmol/L) is proposed, to obtain a high sensitivity but avoid positive results due to unintentional ethanol exposure, e.g. from ethanol-containing mouth wash and hand sanitizer products (Costantino et al., 2006; Rohrig et al., 2006). The DRI-EtG EIA may be applied for routine screening of recent alcohol exposure in clinical and forensic settings.

CONFLICT OF INTEREST DECLARATION

The DRI-EtG EIA kits were provided by Microgenics Corp., but all other costs were covered by the participating laboratories. The authors had complete independence in the interpretation of data and writing of the report.

REFERENCES


