Recent Advances in the Analysis of Ethanol in Saliva: Evaluation of the QED® Device

A.W. Jones and K.Å. Jönsson

Departments of Alcohol Toxicology and Internal Medicine, University Hospital, 581 85 Linköping, Sweden

1. Introduction

Interest in pre-employment and workplace testing for alcohol and drug abuse is evident worldwide (1). When transport-related activities are concerned, alcohol testing becomes a particularly sensitive issue because operator impairment might have contributed to the accident. Obtaining samples of breath, saliva, or urine for analysis of ethanol is less invasive than taking blood samples. Accordingly, much research effort has focused on alternative body fluids for measuring alcohol concentrations and salivary secretions seem to hold much promise as a diagnostic fluid for qualitative and quantitative determinations.

The passage of alcohol from blood into saliva was established during the 1930ies (2). A high correlation between blood-alcohol and saliva-alcohol was demonstrated but saliva did not become popular as a body fluid for alcohol analysis. Wet-chemical methods of alcohol analysis were difficult to work with and required fairly large volumes of specimen. Enzymatic methods for analyzing alcohol in saliva appeared in the 1970ies and with these techniques, micro-volumes (10 μl) were sufficient for each assay (3,4).

This paper deals with the precision, accuracy, and specificity of a new device for measuring alcohol in saliva called QED®. This enzymatic method is well suited for on-site workplace testing for alcohol use. The performance characteristics of the QED® were assessed in controlled experiments under in-vitro and in-vivo conditions.

2. Methods

2.1 Subjects and conditions

Healthy male medical students were recruited for these experiments and
the study was approved by the ethics review board at the University Hospital in Linköping. The subjects drank 0.8 g ethanol/kg body weight within 30 min. This drink was made from ethanol solvent (95% v/v) which was diluted to about 20% v/v with orange juice. Six subjects drank ethanol on an empty stomach after an overnight (10 h) fast and ten others drank the same dose after eating breakfast (full stomach). This experimental design resulted in peak blood-alcohol concentration from 60 to 120 mg/dl and allowed testing the performance of QED® when the composition of saliva might be influenced by eating food. The experiments began at about 08.00 with 2 or 3 subjects taking part in each session. A meal was served 4.5 hours after the drinking began.

2.2 Blood sampling and determination of ethanol

Samples of venous blood were obtained through an indwelling catheter at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, and 420 min after the start of drinking. The catheter tubing was flushed with heparin/saline between the times of obtaining successive blood specimens. The blood was drawn into 5 ml Vacutainer tubes (Becton Dickinson, USA) containing sodium fluoride (20 mg) and heparin 75 units as the preservatives. The concentrations of ethanol were determined in duplicate by headspace gas chromatography as described in detail elsewhere (5). The standard deviation (SD) of this gas chromatographic method was 0.6 mg/dl at a mean BAC of 80 mg/dl.

2.3 Determination of ethanol in breath

The concentration of ethanol in breath was determined in duplicate with an infrared analyzer, BAC Datamaster. Each subject took a moderate inhalation and then exhaled into the breath analyzer according to the recommended procedure. Breath testing began 15 min after the end of drinking and was repeated each time that blood was drawn. The precision of breath alcohol analysis with the Datamaster instrument was 1.7 mg/dl BAC equivalent at a mean BAC of 54 mg/dl.

2.4 Sampling and analysis of saliva with QED®

The QED® is manufactured by Enzymatics Incorporated, Horsham, PA, USA. The principle of operation is enzymatic oxidation with alcohol dehydrogenase and after various side reactions, a colored endpoint is produced. The result of a test starts to develop after about 30 s and is completed after 2 min. Each QED® is individually packed and comprises a collector stick holding a cotton-wool swab, a reaction capillary tube with a thermometer-like scale, instructions for use, and a pouch of silica-gel
desiccant. A novel feature of the QED® is a built-in quality control check called the QA Spot™ which serves to validate completion of each test. Reliable results with the QED® requires that the capillary tube fills completely with saliva. The concentration of ethanol in the sample is then read from the scale on the tube after 2 minutes. Readings can be made to within ± 5 mg/dl. The first samples of saliva were obtained 15 minutes after end of drinking.

2.5 In-vitro experiments

The response of QED® was tested with aqueous solutions of ethanol, methanol, isopropanol, n-propanol, methyl ethyl ketone, acetone, and ethylene glycol. These solutions were prepared from the pure solvents supplied by E. Merck Ltd, Darmstadt, Germany and volumetric dilutions with distilled water were made to give a target concentration of 100 mg/dl. The accuracy and precision of QED® was tested in-vitro under blind conditions by making 20 determinations with solutions containing 0, 50, 100 and 140 mg/dl. These were prepared from absolute ethanol by weighing and volumetric dilution. The strength of these standards was verified by gas chromatography after calibrating the instrument with ethanol standards (50, 100, and 150 mg/dl) supplied by E. Merck.

3. Results

3.1 In-vitro experiments

The QED® gave no response to aqueous solutions (100 mg/dl) of methanol, ethylene glycol, acetone, or methyl ethyl ketone. However, n-propanol showed 60 mg/dl "apparent ethanol" and isopropanol also reacted but took longer than 2 min to reach an endpoint. The precision of QED® expressed as coefficient of variation was between 3 and 4% (N=20) at ethanol concentrations of 50, 100, and 140 mg/dl. The accuracy of QED® expressed as the mean recovery from target values ranged from 97 to 102%.

3.2 In-vivo experiments

Figure 1 (upper part) shows the mean venous blood-ethanol profiles after subjects drank 0.8 g ethanol/kg body weight on an empty stomach. The corresponding saliva-ethanol profiles derived from tests with QED® are shown for comparison. Mean saliva-alcohol (QED®) and breath alcohol profiles (Datamaster) are plotted in the lower part of figure 1. The general shapes of these ethanol profiles agree well regardless of the body fluid analyzed. When ethanol was ingested after breakfast the concentration
time relationships followed a lower time course but the inter-relationships between saliva-alcohol and venous BAC and saliva-alcohol and BrAC were similar to those in figure 1.

Fig. 1. Concentration-time profiles of ethanol in venous blood and saliva (upper trace). The lower trace shows blood alcohol values estimated by analysis of breath with Datamaster.

Figure 2 is a scatter plot of saliva alcohol concentration determined using the QED® and venous blood alcohol analyzed by gas chromatography. A strong linear association is obvious ($r = 0.96$). A high correlation ($r =$
0.97) was also observed between saliva-alcohol (QED®) and breath-alcohol (Datamaster). The concentrations of ethanol in saliva were higher than those in venous blood as indicated by the regression coefficient exceeding unity. The QED® device has a cut-off at 10-15 mg/dl and concentrations of ethanol below this threshold BAC gave zero readings. Table 1 shows the mean and 95% limits of agreement between saliva-alcohol and blood-alcohol and saliva-alcohol and breath-alcohol.

Table 1. Mean and 95% limits of agreement between saliva-alcohol (QED®), venous BAC (gas chromatography) and BrAC (Datamaster).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean diff ± SE</th>
<th>95% limits of agreement</th>
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<tbody>
<tr>
<td>Saliva - Venous BAC</td>
<td>4.4 ± 0.91 mg/dl$^1$</td>
<td>-18 to +26 mg/dl</td>
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<tr>
<td>Saliva - BrAC</td>
<td>3.3 ± 0.71 mg/dl$^1$</td>
<td>-12 to + 19 mg/dl</td>
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$^1$ Statistically different from zero.

Fig. 2. Scatter plot of venous blood-alcohol analyzed by gas chromatography and saliva-alcohol analyzed with QED®.
4. Discussion

The involvement of alcohol in accidents at work and on the highway is a major issue and concern for government action. Testing for alcohol is recommended in emergency medicine if a patient's breath smells of alcohol or if he or she seems confused and disoriented. The diagnosis of skull trauma is complicated if patients are under the influence of alcohol. Alcohol testing at detoxification and correctional units is necessary if the inmates must refrain from drinking. There is clearly a need for fast and reliable ways to test for alcohol in many different situations.

The in-vitro tests with QED® confirmed the high precision and accuracy of this device over an ethanol concentration range from 0 to 140 mg/dl. The selectivity of QED® for ethanol was good as expected for enzymatic (alcohol dehydrogenase) methods of analysis. However, the in-vivo time profiles of ethanol concentration in saliva and whole blood were not identical. Saliva contains more water and therefore more alcohol than an equal volume of blood. Moreover, saliva-alcohol concentrations run closer to the arterial blood concentrations (6). Arterio-venous differences will influence the relationship between saliva-alcohol and venous blood-alcohol. Breath-alcohol is closer to the arterial blood concentration and should therefore show a better agreement with the QED® results as found in this study. Table 1 shows that the mean difference between saliva and blood-alcohol and saliva and breath-alcohol were less than 5 mg/dl.

Until recently, on-the-spot tests for alcohol involvement were made using various kinds of breath-alcohol analyzer. But saliva is an alternative body fluid for alcohol analysis and the QED® has great potential as a practical method for on-site testing and control of post-accident alcohol involvement. Compared with most breath-alcohol analyzers, the QED® enzymatic method has enhanced selectivity for ethanol. Accordingly, a breath alcohol test in conjunction with a QED® saliva alcohol test furnishes a useful testing strategy in emergency medicine when patients might have ingested methanol. Determination of ethanol in saliva with QED® showed no false positive results at the zero level. Tests with QED® were easy to perform and calibration checks are unnecessary each time the device is used.

5. References


