Evaluation of an Onsite Alcohol Testing Device for Use in Postmortem Forensic Toxicology*

David A. Engelhart† and Amanda J. Jenkins
The Office of Cuyahoga County Coroner, 11001 Cedar Road, Cleveland, Ohio 44106

Abstract

The disposable QED® saliva alcohol test provides a very simple, fast, and reliable means for quantitative onsite alcohol detection. The purpose of this study was to determine if the QED test would be a useful tool for the determination of postmortem ethanol levels in cases where a rapid result was needed. QED results were compared with ethanol levels determined by headspace GC analysis. Both saliva and vitreous humor specimens were used for the evaluation. QED tests were initially attempted using the oral fluid from 50 individuals. Of these cases, 17 of the tests were valid with 8 positive results. For 23 cases the oral fluid was not attainable, and for 10 cases the sample was contaminated with blood making the tests invalid. The correlation between the oral fluid results and the blood headspace GC analysis was poor ($r = 0.8345$) over the range of 0.01–0.29 g/dL. Vitreous specimens were found to be the matrix of choice for analyzing postmortem cases using the QED. Only 6 of 171 specimens were found to be unsuitable. The QED results correlated well with the headspace GC analysis ($r = 0.9931$, $n = 165$). When using ethanol levels > 0.02 g/dL ($n = 126$), an average vitreous (GC)/blood ratio of 1.16 correlated well with the average QED/blood ratio of 1.22.

Although the QED saliva alcohol test does not appear to be useful in determining postmortem saliva ethanol levels, it does provide accurate results when using postmortem vitreous humor as the testing matrix.

Introduction

As a result of the prevalence of alcohol use in our society and its involvement in accidents and deaths, rapid techniques for the analysis of ethanol have become more popular and necessary. Whereas the relationship between saliva- and blood-ethanol levels was demonstrated as early as 1938 (1), the use of saliva as a testing matrix for ethanol did not gain much attention until the last 10 years when rapid onsite tests for alcohol in saliva have been marketed (2–4). The relationship of postmortem saliva- and blood-ethanol levels, however, has not been investigated for obvious reasons: oral fluid is not routinely obtained at autopsy; saliva is not the specimen of choice in postmortem ethanol analysis; and contamination of the oral cavity by blood in trauma cases and by gastric fluid has limited its usefulness.

Vitreous humor is considered to be an ideal specimen for postmortem ethanol analysis. It does not contain glucose or microorganisms that can lead to postmortem ethanol production (5). The vitreous is also afforded some protection from putrefaction or trauma and can be a useful specimen to analyze for ethanol in embalmed cases (6,7). The vitreous is also a postmortem specimen can be obtained easily and rapidly. Several studies have reported the correlation between vitreous humor- and blood-ethanol concentrations (8). For these reasons, the vitreous humor was chosen as an alternate matrix for evaluating the usefulness of the disposable QED (STC Technologies, Bethlehem, PA) saliva-alcohol testing device to provide a fast and accurate estimation of blood-ethanol levels in postmortem cases.

The QED saliva alcohol test provides a very simple, fast, and reliable means for quantitative onsite alcohol detection in living individuals. The test is based on the enzymatic oxidation of ethanol by alcohol dehydrogenase. Saliva is collected from the mouth using a cotton swab collector. Once the swab is saturated, the collector is inserted into the entry port of the test device. The alcohol concentration can be read from the color bar. Each test has a built-in quality-control check called a QA Spot™ that indicates whether the test was properly performed and therefore considered valid. Tests are individually packaged and available in two test ranges. The QED A150 and QEDA350 measure from 0.01 to 0.15 g/dL and 0.01 to 0.35 g/dL, respectively. In clinical trials, saliva-alcohol levels measured by the QED device demonstrated high correlation with blood-, urine- and breath-alcohol levels (4,9–14).

The objective of this study was to determine whether the QED device would be a useful tool for the determination of postmortem ethanol levels in cases where a rapid result was needed. Results were compared with headspace gas chromatography (GC). Both saliva (oral fluid) and vitreous humor specimens were used for the evaluation.

Materials and Methods

Materials

QED saliva alcohol tests were obtained from STC Technologies. Ethanol, 200 proof, was obtained from Spectrum Chemical Mfg.
Corp. (Gardena, CA). n-Propanol was obtained from Burdick and Jackson Laboratories, Inc. (Muskegon, MI).

Instrumentation
A Hamilton MicroLab-500 pipettor-diluter was used to aliquot specimens for GC-headspace analysis. Specimens were analyzed using a PE-8500 GC equipped with a flame ionization detector and an HS-101 headspace autosampler. The column was a Restek stainless steel column (1.8 m x 2-mm i.d., 1/8-in. o.d.) packed with 5% Carbowax 20M on 60/80 CarboBlack (Restek Corporation, Bellefonte, PA).

Specimens
Oral fluid specimens were obtained from individuals with suspected alcohol-related deaths or who had a history of ethanol abuse. All autopsies were performed at the Office of the Cuyahoga County Coroner, Cleveland, OH. Oral fluid specimens were obtained before autopsy from the oral cavity using the QED device and tested immediately. Vitreous specimens were obtained at autopsy in red-top Vacutainers® and stored at 4°C until analysis. QED analysis was performed on vitreous specimens for the cases in which the vitreous and/or blood specimens were determined to be positive for ethanol (LOQ = 0.01 g/dL) by headspace GC analysis. Collector swabs were dipped into approximately 0.5 mL of vitreous humor until saturated. No sample preparation such as centrifugation/filtration was performed.

Results
Oral fluid
QED tests were initially attempted using the oral fluid from 50 individuals. Of these cases, 17 of the tests were valid and 8 were positive. For 23 cases, the oral cavities were too dry to obtain enough oral fluid to perform a valid test. For 10 cases, the sample was contaminated with blood, rendering the tests invalid. The correlation between the oral fluid results and the heart blood analyzed by headspace GC was poor ($r = 0.8345$) over the range of 0.01–0.29 g/dL ($n = 10$).

Vitreous humor
Vitreous humor specimens were found to be the matrix of choice for analyzing postmortem cases using the QED. Of the 171 specimens analyzed, only 6 were found to be invalid because of the inability of the fluid to travel the length of the device in order to turn the QA Spot purple. The analysis could be performed on as little as 0.25 mL of vitreous humor. The QED results correlated well with headspace GC analysis of the same samples ($r = 0.9931$, $n = 165$, Figure 1). When using ethanol levels > 0.02 g/dL ($n = 126$), an average vitreous (GC)/blood ratio of 1.16 was similar to an average vitreous (QED)/blood ratio of 1.22. These results corroborated previously reported average vitreous/blood ratios ranging from 0.90 to 1.38 (8). Vitreous (GC)/blood and QED/blood ratios of 1.24 and 1.32, respectively, were determined when ethanol levels 0–0.35 g/dL were used in the calculation ($n = 139$).

Analytical sensitivity
For all analyses, the cutoff concentration for a positive result was 0.01 g/dL. The number of true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN) was determined by comparison of the QED result with the GC result. A sample was considered a true positive if both the QED and GC results were both positive for ethanol or a true negative if both were negative for ethanol. A false-positive result was one in which the QED result was positive but the GC result was negative. Samples for which the QED result was negative but the GC result was positive were considered to be false negatives. Sensitivity was calculated according to the following formula (15): sensitivity = $TP/(TP + FN)$.
In evaluating the sensitivity of the QED device, the data was divided into three separate categories. The first category included the oral fluid results, obtained by QED analysis, and the GC blood results. Because the QED device did not perform well with the measurement of postmortem saliva-ethanol levels, sensitivity issues for this group are not relevant. The second category included the QED vitreous and the GC vitreous results. At a concentration of 0.01 g/dL, the analytical sensitivity was 91.7% (n = 12) and at concentrations ≥ 0.02 g/dL, the sensitivity was ≥ 95.5%. There were no false positives and only one false negative for this group. The overall sensitivity over the range of 0.01–0.35 g/dL was 99.4% (n = 164). The third group of data evaluated was the volatile substances measured in postmortem specimens are acetone, methanol, isopropanol, and ethylene glycol. These substances were further evaluated in this study (see Table 1).

When comparing the QED vitreous and GC vitreous results, at concentrations < 0.04 g/dL, the analytical sensitivity was 100% (n = 44). Therefore, the decrease in sensitivity, when comparing the QED vitreous and GC blood results, at concentrations ≤ 0.04 g/dL was due to a matrix effect and not the sensitivity of the QED device. The overall sensitivity over the concentration range of 0.01–0.35 g/dL was 99.2% (n = 140).

Analytical specificity

The specificity of the QED device was evaluated by the manufacturer. The substances listed in Table 1 do not interfere with the test results at the concentrations indicated (i.e., did not produce a positive result) (16). In addition to ethanol, the most common volatile substances measured in postmortem specimens are acetone, methanol, isopropanol, and ethylene glycol. These substances were further evaluated in this study (see Table 1 experimental concentrations). The QED test was performed on aqueous solutions of increasing concentration. Acetone and methanol did not interfere with the QED test at concentrations up to 640 mg/dL. Isopropanol gave positive results corresponding to 0.01, 0.02, and 0.03 mg/dL of ethanol at concentrations of 20, 40, and 80 mg/dL, respectively. Ethylene glycol did not interfere with the analysis at concentrations below 80 mg/dL.

**Table 1. Substances Interfering with the QED Saliva Alcohol Testing Device**

<table>
<thead>
<tr>
<th>Compound</th>
<th>STC Reported Concentrations (mg/dL)*</th>
<th>Experimental Concentrations (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene glycol</td>
<td>17.5</td>
<td>80</td>
</tr>
<tr>
<td>Acetone</td>
<td>70</td>
<td>&gt;640</td>
</tr>
<tr>
<td>Methanol</td>
<td>70</td>
<td>&gt;640</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>7.0</td>
<td>n.d.†</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>35</td>
<td>n.d.</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>7.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>35</td>
<td>n.d.</td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>7.0</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>3.5</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* QED saliva alcohol test package insert 10341, Rev. 4/98, STC Technologies, Bethlehem, PA.
† not determined.

**Discussion**

Although the ability of the QED saliva alcohol test to accurately measure clinical saliva-ethanol levels and its correlation to blood-ethanol levels has been well documented (4,8–14), this is the first reported study evaluating the QED device in the postmortem setting. The results demonstrate that postmortem saliva/oral fluid-ethanol levels are very difficult to obtain because of insufficient or contaminated samples. Therefore, in most cases, postmortem oral fluid will not provide a reliable estimate of blood ethanol levels. However, the ability to determine accurate postmortem vitreous-ethanol levels with the QED device was demonstrated by comparison to GC results (Figure 1). Over the concentration range of 0.01–0.35 g/dL, the QED device provided to be as sensitive and accurate as headspace GC analysis. Use of vitreous humor specimens for the analysis was also found to be reliable, since only 6 of the 171 tests performed were aborted because of the high viscosity of the specimen.

When using the QED device, interference due to other substances commonly found in postmortem specimens must be considered. In contrast to the values reported by STC Technologies (16), acetone and methanol did not interfere with the analysis at concentrations up to 640 mg/dL. Isopropanol and ethylene glycol, however, may interfere with the test at concentrations as low as 20 and 80 mg/dL, respectively, and must be taken into consideration when performing the analysis.

Because this study has demonstrated that the QED device will provide accurate measurement of postmortem vitreous-ethanol levels, an estimated blood-ethanol level may be obtained. However, several factors must be considered when performing this calculation. As shown in Figure 2, at vitreous-ethanol concentrations less than 0.05 g/dL, the blood was negative for ethanol in more than 40% of the cases. This is independent of the method of analysis of the vitreous specimen. Therefore, the use of an average vitreous/blood ratio to estimate blood ethanol levels from measured vitreous levels is only valid at blood-ethanol concentrations exceeding 0.04 g/dL. In most instances where a rapid ethanol result may be needed to determine the involvement of alcohol in a death, low concentrations will probably be of limited utility.

In conclusion, the QED saliva alcohol test does not appear to be useful in determining postmortem saliva-ethanol levels because of the inability to obtain a sufficient or uncontaminated sample. However, it does provide accurate results when using postmortem vitreous humor as the testing matrix. Using an average vitreous/blood ethanol ratio of 1.2 for concentrations > 0.04 g/dL, the QED test may be a useful tool for the estimation of postmortem blood-ethanol levels in cases where a rapid result is needed.

**Acknowledgment**

The authors thank OraSure Technologies, formerly STC Technologies, Inc., Bethlehem, PA, for providing the QED Saliva Alcohol Tests for this project.
References


16. Q.E.D. Saliva Alcohol Test package insert 10341, Rev. 4/98, STC Technologies, Bethlehem, PA.