TECHNICAL NOTE

Constance M. Senkowski,[1] *B.S. and*
Kimberly A. Thompson,[1] *B.S.*

The Accuracy of Blood Alcohol Analysis Using Headspace Gas Chromatography When Performed on Clotted Samples

REFERENCE: Senkowski, C. M. and Thompson, K. A., "**The Accuracy of Blood Alcohol Analysis Using Headspace Gas Chromatography When Performed on Clotted Samples,**" *Journal of Forensic Sciences,* JFSCA, Vol. 35, No. 1, Jan. 1990, pp. 176–180.

ABSTRACT: Subjects consumed alcoholic beverages and attained blood ethyl alcohol concentrations ranging from 0.02 to 0.15 g/dL. Sets of blood samples were drawn from these subjects, including some samples that were allowed to clot and some in which anticoagulent was added. A quantitative analysis for ethyl alcohol was performed on these samples using headspace gas chromatography. The mean deviation of the concentration of ethyl alcohol in the clotted samples from the ethyl alcohol concentration in the corresponding control samples was 0.001 g/dL. The 99% confidence interval for this mean was ± 0.0005 g/dL.

KEYWORDS: criminalistics, blood, alcohol, chromatographic analysis

In the State of Florida, the agency that administers the program for licensing individuals to do blood alcohol analysis and delineates the procedures for performing this analysis is the Department of Health and Rehabilitative Services. Their regulations require that this analysis be performed only on unclotted samples. A large number of the samples received by the Florida Department of Law Enforcement, Jacksonville Regional Crime Laboratory for analysis are clotted. The purpose of this study was (1) to prove that if the sample is properly handled an accurate analysis can be done on a clotted sample and (2) to calculate the limits of that accuracy.

Method

A group of subjects consumed alcoholic beverages and attained blood ethyl alcohol concentrations ranging from 0.02 to 0.15 g/dL. A set of four tubes of blood was drawn from each subject at two different times. Each set of four tubes included two that contained an anticoagulant and were thoroughly mixed (control), one that contained an anticoagulant but was not mixed, and one that did not contain an anticoagulant. Blood,

Received for publication 6 July 1988; revised manuscript received 13 Jan. 1989; accepted for publication 28 Feb. 1989.

[1]Crime laboratory analysts, Florida Department of Law Enforcement, Jacksonville Regional Crime Laboratory, Jacksonville, FL.

which does not contain an anticoagulant, will clot. Often, however, tubes containing anticoagulant, which are not mixed sufficiently, will also clot to a lesser extent. Preparation of the clotted samples in the two ways described above allowed for evaluation of semiclotted versus completely clotted samples. All of the tubes used were Becton and Dickinson Vacutainer® tubes. Some tubes were 10-mL capacity, containing 10 mg of disodium edetate (EDTA) and 20 mg of sodium fluoride. Some tubes were 7-mL capacity, containing 7 mg of disodium EDTA and 17.5 mg of sodium fluoride. All tubes of blood were refrigerated at 4°C and analyzed within three weeks of being drawn. The analysis of the blood was done by gas chromatography. Clotted samples were ground in a Pyrex® Ten Broeck tissue grinder to allow accurate sampling. One millilitre of blood was delivered into a test tube using an Oxford P-7000 pipet. One millilitre of a normal propanol solution was placed in this test tube using a LABINDUSTRIES repipet. The tube was capped with a Wheaton rubber stopper and placed in a 30°C water bath for 45 min. A 1-mL sample of headspace was injected in the GOWMAC 750 gas chromatograph equipped with a flame-ionization detector. The 6-ft (2-m) glass column was packed with 80–100 mesh Porapak Q and used isothermally at 160°C. Nitrogen was used as the carrier gas with a flow rate of 54 mL/min.

Results

The data, resulting from the analysis of blood from 20 sets of tubes, is listed in Table 1 (Columns A, B, and C). Duplicate analysis was performed on samples from three tubes for each sample set: one of the control tubes (Column A, Table 1), the tube containing no anticoagulant (Column B, Table 1), and the tube containing anticoagulant but not properly mixed (Column C, Table 1). The remaining columns of Table 1 are the result of the evaluation of the data.

The maximum amount that any clotted sample deviated from the blood alcohol concentration in its corresponding control was 0.006 g/dL (Set 19, Table 1). This represented a relative deviation of 4.6% as the blood alcohol concentration was 0.133 g/dL. The maximum amount that any clotted sample was greater than the blood alcohol concentration in its corresponding control was 0.001 g/dL (Set 1, Table 1). This represented a relative deviation of 4.5% as the blood alcohol concentration was 0.022 g/dL.

A standard curve was prepared using aqueous ethanol before analysis of the experimental blood samples to determine the accuracy of this system of analysis. Nine standards, ranging in concentration from 0.020 to 0.200 g/dL, were prepared and analyzed by the same method described above for the experimental blood samples. The relative deviation of the ethyl alcohol concentration, determined by this method of analysis, and the known concentration of each standard ranged from 0 to 5.2%. In no sample in which the ethyl alcohol concentration was greater than 0.050 g/dL was this relative deviation greater than 5%. The relative deviation between the ethyl alcohol concentration of the clotted blood samples and their corresponding unclotted samples ranged from 0 to 11%. In no sample in which the ethyl alcohol concentration was greater than 0.050 g/dL was this relative deviation greater than 5%.

The difference between the concentration of the control and the concentration in the tube containing no anticoagulent was calculated for each set of tubes (Column D, Table 1). The difference between the concentration of the control and the concentration of the tube containing anticoagulant, but not properly mixed, was calculated for each set of tubes (Column E, Table 1). The mean for the values listed in Column D, Table 1 was calculated to be 0.001 g/dL. The 99% confidence interval on this mean was calculated to be ± 0.0005 g/dL. The mean for Column E, Table 1 was calculated to be 0.001 g/dL. The 99% confidence interval on this mean was also calculated to be ± 0.0005 g/dL.

A plot of the ethyl alcohol concentration of each clotted sample in which there was

TABLE 1—*Ethyl alcohol (ETOH) concentrations (g/dL) of control versus clotted blood specimens.*

Set	(A)[a] Control Conc.	(B)[b] Clotted Conc.	(C)[c] Clotted Conc.	(D)[d] |A-B|	(E)[e] |A-C|
1	0.022	0.023	0.022	0.001	0
	0.022	0.023	0.022	0.001	0
2	0.033	0.032	0.032	0.001	0.001
	0.032	0.032	0.032	0	0
3	0.033	0.033	0.033	0	0
	0.033	0.033	0.032	0	0.001
4	0.036	0.035	0.034	0.001	0.002
	0.036	0.035	0.034	0.001	0.002
5	0.038	0.037	0.035	0.001	0.003
	0.037	0.037	0.036	0	0.001
6	0.039	0.038	0.040	0.001	0.001
	0.039	. . .	0.040	. . .	0.001
7	0.045	0.044	0.041	0.001	0.004
	0.044	0.043	0.040	0.001	0.004
8	0.064	0.064	0.064	0	0
	0.064	0.065	0.064	0.001	0
9	0.066	0.064	0.064	0.002	0.002
	0.065	0.063	0.064	0.002	0.001
10	0.072	0.073	0.072	0.001	0
	0.072	0.072	0.072	0	0
11	0.075	0.076	0.075	0.001	0
	0.075	0.075	0.076	0	0.001
12	0.087	0.088	0.086	0.001	0.001
	0.089	0.087	0.085	0.002	0.004
13	0.091	0.088	0.091	0.003	0
	0.090	0.088	0.089	0.002	0.001
14	0.099	0.099	0.098	0	0.001
	0.099	0.100	0.098	0.001	0.001
15	0.102	0.099	0.103	0.003	0.001
	0.101	0.098	0.100	0.003	0.001
16	0.109	0.108	0.108	0.001	0.001
	0.108	0.108	0.108	0	0
17	0.109	0.109	0.107	0	0.002
	0.109	0.108	0.106	0.001	0.003
18	0.133	0.131	0.130	0.002	0.003
	0.133	0.131	0.131	0.002	0.002
19	0.133	0.127	0.131	0.006	0.002
	0.130	0.131	0.129	0.001	0.001
20	0.141	0.141	0.137	0	0.004
	0.140	0.141	0.139	0.001	0.001

[a] Column A: ETOH concentrations for control tubes (duplicate analysis of each tube).
[b] Column B: ETOH concentrations for tubes containing no anticoagulant (duplicate analysis of each tube).
[c] Column C: ETOH concentrations for tubes containing anticoagulant but not properly mixed (duplicate analysis of each tube).
[d] Column D: Difference in concentration (g/dL) between control and clotted tube with no anticoagulant (|Column A-Column B|).
[e] Column E: Difference in concentration (g/dL) between control and clotted tube with anticoagulant but not properly mixed (|Column A-Column C|).

no anticoagulant present versus the concentration of its corresponding control is shown in Fig. 1 (Column B, Table 1 versus Column A, Table 1). The equation for the regression line calculated from this data is $y = 0.9898x + 9.6592 \times 10^{-5}$. The correlation coefficient is 0.9992. A plot of the ethyl alcohol concentration of each clotted sample in which there was anticoagulant present but the tube was not properly mixed versus the concentration of its corresponding control is shown in Fig. 2 (Column C, Table 1 versus Column A, Table 1). The equation of the regression line calculated from this data is $y = 1.0077x + 5.4456 \times 10^{-4}$. The correlation coefficient is 0.9993. A 99% prediction interval was calculated for each regression line. In each case this prediction interval was equal to the

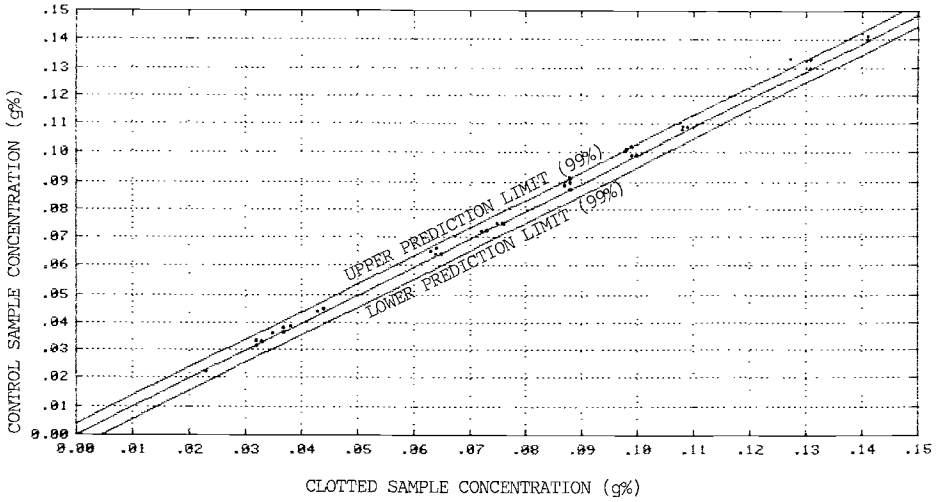

FIG. 1—*Plot of control ETOH concentration versus clotted ETOH concentration for tubes with no anticoagulant.*

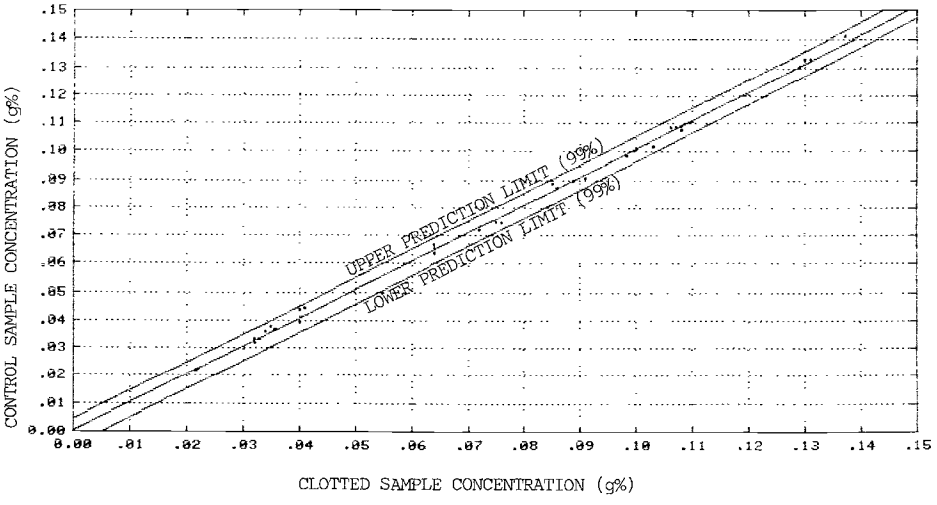

FIG. 2—*Plot of control ETOH concentration versus clotted ETOH concentration for tubes containing anticoagulant but not properly mixed.*

linear regression estimate of y (the control sample concentration) $\pm$ 0.004 g/dL. These prediction limits are plotted on Figs. 1 and 2.

Discussion and Conclusions

The data demonstrate that a valid determination of ethyl alcohol content can be performed on a clotted blood sample. Some of the deviation between clotted and nonclotted concentration may be attributed to the accuracy of the system of analysis and the thoroughness of the homogenization process. The tendency for the ethyl alcohol concentration to be slightly lower in the clotted sample compared to the concentration determined in the control is probably due to the grinding process used in analyzing the clotted samples. To grind the sample, it must be poured into the tissue grinder. After the grinding process, the sample is then poured back into the test tube. During the transfer of sample there is exposure of more sample surface area, allowing the escape of volatiles, including ethyl alcohol.

Acknowledgments

The planning and execution of this research was centered in the Toxicology Section of the Florida Department of Law Enforcement, Jacksonville Regional Crime Laboratory, but the efforts of the personnel from the Department of Health and Rehabilitative Services, the participating local law enforcement agencies, and the other FDLE lab personnel were all critical to the success of the research.

Address request for reprints or additional information to
Constance M. Senkowski
Florida Department of Law Enforcement
Jacksonville Regional Crime Laboratory
711-A Liberty St.
Jacksonville, FL 32202