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Evaluation of the Abbott TD_x-Radiative Energy Attenuation (REA) Ethanol Assay in a Study of 1105 Forensic Whole Blood Specimens

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ABSTRACT: In a preliminary study to determine the applicability of the Abbott radiative energy attenuation (REA) method for the quantification of ethanol in whole blood specimens it was concluded that a larger number of samples was required to evaluate the method, particularly for use in forensic toxicology applications. In this study, 573 blood specimens from suspected driving while intoxicated individuals (DWI blood) and 532 postmortem blood specimens (PM blood) were analyzed by the REA method and a headspace gas chromatographic method (GC) currently used in this laboratory. "Negative" specimens (<10 mg/dL by GC) and "positive" specimens (≥ 10 mg/dL by GC) in each category were analyzed. Linear regression analysis comparing the REA values with the GC values was performed for each type of blood specimen. The equation obtained for DWI blood specimens was REA = 0.943 GC + 1.54; the equation for PM blood specimens was REA = 0.980 GC + 2.76. The correlation coefficient for each group was greater than 0.99. The data suggested that a limit of detection of 10 mg/dL could be applied for DWI blood specimens, while 20 mg/dL would be recommended as the limit of detection for PM blood specimens.

KEYWORDS: toxicology, alcohol, blood, gas chromatography, forensic blood specimens, enzyme immunoassay

In a previous report [*1*], a preliminary study evaluating 2 Abbott radiative energy attenuation (REA) methods for the quantification of ethanol in serum, urine, and whole blood was presented. Specifically, 2 REA assays differing only in the dye used to generate the chromophore (TD_x-INT and TD_x-MTT) were compared with a headspace gas chromatographic (GC) method used in this laboratory for the quantification of ethanol in biological specimens. As a result of the limited number of specimens tested, the study concluded that a larger population of specimens was required to assess better the applicability of this assay to whole blood ethanol analysis. It appeared that of the 2 methods, the more recently developed TD_x-MTT assay gave more accurate results for blood ethanol analysis. Therefore, this report describes the more comprehensive study undertaken to compare the TD_x-MTT assay with the GC assay. A criterion of a minimum of 500 whole blood specimens of each of 2 types was established. This included blood from suspected driving while intoxicated drivers (DWI blood) and postmortem blood (PM blood). The results are presented as well as recommendations for the use of the REA ethanol assay for forensic toxicology purposes.

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Experimental Procedure

Specimen Acquisition

Whole blood specimens from suspected driving while intoxicated individuals (DWI blood) were obtained from the Maryland State Police and the University of Maryland Hospital. Postmortem blood specimens were obtained from the Maryland Medical Examiner's Office.

REA Ethanol Analysis

The Abbott TD_x analyzer was used for the REA ethanol analysis. At the beginning of the study, the instrument was calibrated using aqueous calibrators supplied by Abbott for use with the TD_x. The instrument was recalibrated only when a new lot of reagents was used. The instrumental parameters used were as described in the Abbott System Assays manual for the TD_x [*2*].

A set of 3 serum controls was included at the beginning and end of each carousel of 20 samples. These controls had approximate concentrations of 40, 100, and 250 mg/dL and were also supplied by Abbott. Each blood specimen was tested in singlicate. Since the assay is calibrated only to 300 mg/dL, any sample with a greater concentration was repeated using the dilution protocol as described in the System Operation Manual [*3*].

Gas Chromatographic Analysis

The Perkin-Elmer F-45 Headspace Analyzer attached to a Sigma I data station was used as the reference alcohol method. Temperature control was as follows: bath temperature, 60°C; injector temperature, 120°C; oven temperature, 105°C; and detector temperature, 200°C. Nitrogen was the carrier gas flowing at 28 mL/min. The injector needle was exposed to the vapor of each sample for 6 s. The column was 0.02% Carbowax 1500 on 80-100 mesh Carbopack C. Samples were prepared by diluting 0.5 mL of blood or standard to 5.0 mL with the internal standard (20-mg/dL *n*-propanol in water). This dilution was performed using a Dilumat® (Fisher). The samples were then placed in the instrument bath and equilibrated for at least 30 min before injection. At the beginning of each run, the instrument was calibrated using a 250-mg/dL aqueous standard whose concentration was determined using dichromate oxidation. During a run, every tenth sample was the standard.

Results

To determine whether any differences in the calibration of the two methods existed, the REA calibrators were analyzed by the GC method and the GC calibrator was analyzed by the REA method. Since the REA method requires that the sample be a conducting solution and the GC method uses an aqueous calibrator, 5 mg of sodium chloride per millilitre of solution was added to the GC calibrator before REA assay. The results are shown in Table 1. No differences greater than 6% were noted in this calibrator crossover study.

The experimental design called for the simultaneous analysis of at least 500 specimens, with a minimum of 250 "positive" specimens and 100 "negative" specimens. "Negative" specimens were defined as those having a GC value of less than 10 mg/dL; "positive" specimens had a GC value greater than 10 mg/dL. When reporting ethanol values, 10 mg/dL is the cutoff used in this laboratory. A difference greater than 15% between the TD_x method and the GC method or a concentration greater than 10 mg/L by 1 method and less than 10 mg/L by the other method was considered a discrepancy in results which required reanalysis of the specimen by each method.

In this study, 573 DWI blood specimens were tested, 100 negative specimens and 473

TABLE 1—*Calibrator crossover study.*

TD_x Calibrator Concentration, mg/dL	Average GC Concentration Obtained (mg/dL; $N = 2$)	GC Standard Concentration, mg/dL	TD_x Concentration Obtained (mg/dL; $N = 2$)
0	0.3 (0.0,0.6)	254	242
25.0	23.9 (23.7,24.0)		(242,243)
50.0	47.2 (47.6,46.9)		
100.0	95.7 (96.3,95.0)		
200.0	197.1 (194.1,200.0)		
300.0	299.0 (296.9,301.1)		

positive specimens. A linear regression correlation from these data was generated by plotting the REA values as a function of GC values and is shown in Fig. 1. The equation for the line was REA = 0.943 GC + 1.54. The correlation coefficient for the line was 0.991, thus demonstrating good correlation between the 2 methods. The paired sample difference was 5.5 ± 0.8 mg/dL. Further, the average ethanol concentration of the blood specimens was almost identical by the 2 methods (Table 2). The data were subdivided into concentration ranges to ascertain the variation in correlation between the 2 methods at various ethanol concentrations. The results are shown in Table 3. The total number in agreement after 2 trials is also given in Table 3. Of the 573 DWI specimens analyzed, 547 or 95.5% yielded values within 15% by the 2 methods after 1 trial. When the other 26 were reanalyzed, 18 showed agreement between the 2 methods. Therefore, 565 or 98.6% had values within 15% after 2 trials.

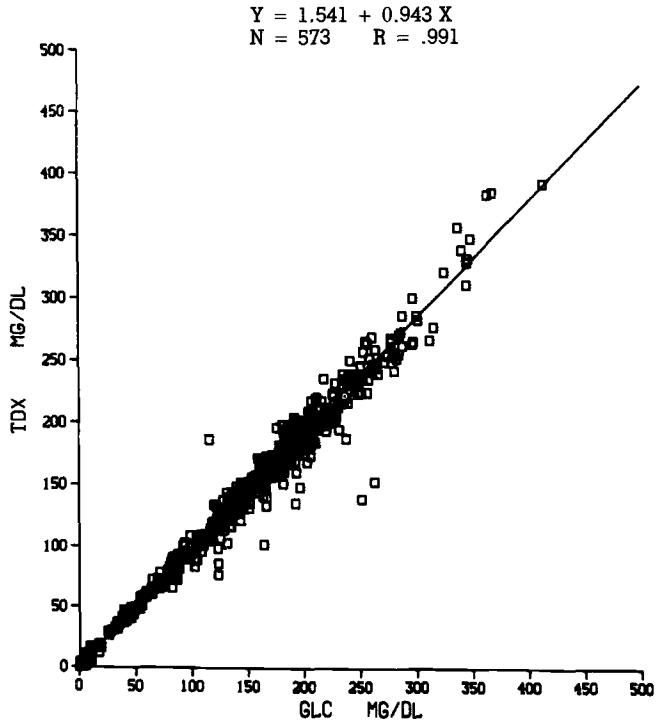

FIG. 1—*Correlation of the REA ethanol assay with gas chromatography for DWI blood specimens.*

TABLE 2—*Average blood ethanol concentrations by the REA and GC methods.*

Specimen Type	Method	Mean ± Standard Deviation, mg/dL
DWI blood	REA	127.6 ± 84.9
	GC	133.6 ± 89.1
PM blood	REA	61.4 ± 90.3
	GC	59.8 ± 91.6

TABLE 3—*DWI blood specimen study results.*

Ethanol Concentration Range, mg/dL	Total Number	Number in Agreement		Percent in Agreement After Two Runs
		After One Run	After Two Runs	
<10	100	97	99	99
10-99	85	77	84	99
100-199	239	228	233	97
200-299	137	133	137	100
≥300	12	12	12	100
Total	573	547	565	98.6

Table 3 illustrates that excellent accuracy was obtained by the REA method throughout the range of ethanol concentrations.

A group of PM blood specimens was also tested by the REA and GC methods. In all, 532 specimens were analyzed; there were 279 negative and 253 positive specimens. The linear regression correlation obtained using the PM blood data produced the equation: REA = 0.980 GC + 2.76. The relationship is presented in Fig. 2. The correlation coefficient was 0.994. The paired sample difference was −1.58 ± 0.83 mg/dL. The average PM blood ethanol concentration was essentially the same by either technique. The subdivision of the data into concentration ranges is given in Table 4. The percentages of samples showing agreement between the TD_x and GC methods for PM blood specimens are somewhat lower than those found with DWI blood samples. This is especially true for ethanol concentrations less than 100 mg/dL. From Table 4, there were 23 "false positives" (GC value less than 10 mg/dL with corresponding TD_x value greater than 10 mg/dL). When these samples were rerun, 20 of the 22 samples (1 sample could not be repeated) still yielded GC values less than 10 mg/dL. However, 12 of the 20 TD_x values remained above 10 mg/dL; 1 of these 12 actually had a value greater than 20 mg/dL. Each of the 2 repeated samples with GC values greater than 10 mg/dL had a TD_x value that was higher than GC by more than 15%. Furthermore, of the 21 samples repeated with concentrations between 10 and 99 mg/dL, 15 had GC and TD_x values less than 50 mg/dL. Therefore, 29 of the 37 specimens that still differed by greater than 15% between the 2 methods after 2 trials had GC and TD_x values less than 50 mg/dL. Hence, it appears that when discrepancies occurred between the TD_x and GC, they were mostly at concentrations less than 50 mg/dL.

Discussion

This study was undertaken to determine the applicability of the Abbott TD_x REA ethanol assay to forensic blood specimens. To effect this evaluation, it was necessary to analyze blood

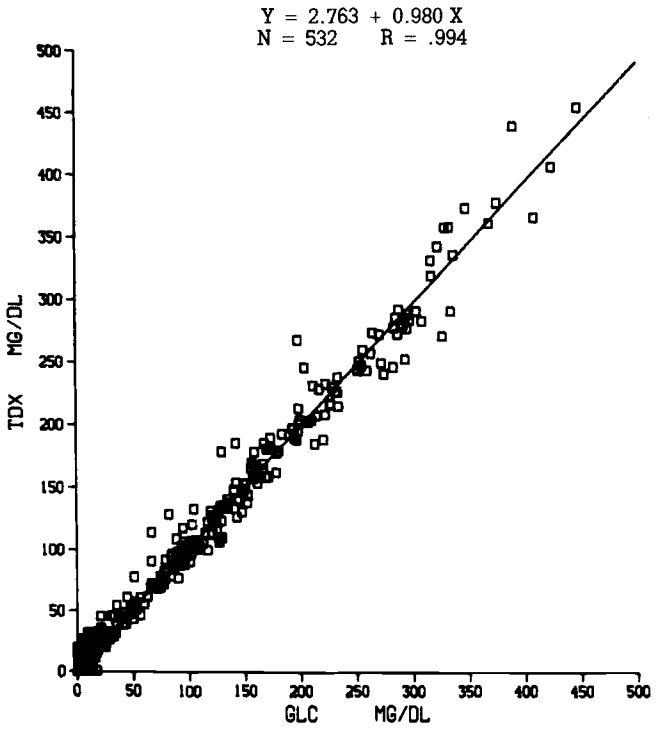

FIG. 2—*Correlation of the REA ethanol assay with gas chromatography for PM blood specimens.*

TABLE 4—*PM blood specimen study results.*

Ethanol Concentration Range, mg/dL	Total Number	Number in Agreement		Percent in Agreement After Two Runs
		After One Run	After Two Runs	
< 10	279	256	264[a]	95
10-99	114	81	92[a]	81
100-199	84	79	82	98
200-299	39	37	39	100
300-399	13	11	13	100
≥ 400	3	3	3	100
Total	532	467	493	92.7

[a]One sample in each group was not repeated because of insufficient volume.

from both living and deceased individuals, since ethanol analyses of both types are frequently required in forensic science settings. It was also imperative that a large number of negative as well as positive ethanol samples be analyzed, because of the fact that the ability to distinguish between positive and negative ethanol concentrations is as important as the concentration determined in positive ethanol cases.

This work clearly showed that the Abbott TD_x REA ethanol assay is an acceptable procedure for the analysis of ethanol in whole blood. Greater than 98% of the DWI blood specimens and 93% of the PM blood specimens produced ethanol concentrations by the REA and

GC methods agreeing within 15% after two trials. Previous work [*1,4*] had demonstrated excellent precision and specificity for the REA ethanol assay.

One of the purposes of this study was to determine the minimum value that can be reported for ethanol by the REA method. This is less critical in the clinical laboratory where values less than 50 mg/dL have little clinical significance. However, in the forensic science laboratory, where the presence or absence of ethanol may be quite significant, it is of great importance that there be reasonable certainty that ethanol was indeed present, even in clinically insignificant concentrations. In this study of the 100 DWI blood specimens whose ethanol concentrations were less than 10 mg/dL by GC, only 1 sample gave a value greater than 10 mg/dL on the TD_x after 2 trials. The TD_x value in this case was 16 mg/dL while the GC value was 9 mg/dL. Therefore, the authors believe that for blood obtained from living individuals, a cutoff of 10 mg/dL may be used for the REA method if all results between 10 and 20 mg/dL are repeated, and the concentration on the repeat analysis is also between 10 and 20 mg/dL. If 1 of the 2 values is less than 10 mg/dL, then the sample should be reported as negative for ethanol.

The cutoff for PM blood specimens is less clear because larger discrepancies at the lower concentrations were observed. Nevertheless, the data suggest that 20 mg/dL is a reasonable cutoff for PM blood specimens. This conclusion was reached from the fact that of all the 279 negative specimens analyzed, in only 2 instances did a value greater than 20 mg/dL appear twice. It is also recommended that all samples less than 50 mg/dL be repeated and the 2 values averaged. If 1 value is less than 20 mg/dL, then the results should be reported as negative for ethanol.

The question arises as to the reasons why the REA and GC methods failed to give comparable results after 2 trials in some cases. The protocol specified reanalysis of any sample which produced a discrepancy between the 2 methods of greater than 15%. Of the 26 DWI blood specimens repeated, 18 gave comparable results the second time, suggesting an error was made which was not inherent in either method. Of the 8 remaining specimens, 7 had consistent intramethod values; that is, the REA method gave essentially the same value each time as did the GC method. This suggests that there were some method specific differences in these specimens. Since no other volatiles were detected, the presence of other volatiles did not provide an explanation. However, in 5 of the 7 samples, the TD_x value was less than the GC value, suggesting some type of fluorescence increasing effect since an increased fluorescence intensity is measured at lower ethanol concentrations. Similar intragroup patterns were not observed with PM blood samples which failed to agree by the 2 methods after 2 trials. In some PM specimens, the REA method values were consistent, in others the results from the GC method were consistent, while in still others, neither or both of the methods showed intramethod consistency between the 2 trials.

The slope and intercept obtained for the regression line relating the two methods compare favorably to similar studies previously published. Poklis and MacKell [*5*] calculated a slope of 0.991 and an intercept of 4.5 mg/dL when comparing a gas chromatographic and an enzymatic method for analyzing ethanol in postmortem blood specimens. Whitehouse and Paul [*6*], when analyzing plasma samples by a gas chromatographic and an enzymatic method, obtained a slope of 1.03 and an intercept of −6.9 mg/dL for the regression line comparing the two methods. A slope of 0.996 and an intercept of 6.5 mg/dL resulted from the data of Jones et al. [*7*] who analyzed blood serum by both gas chromatographic and enzymatic methods. Although the above illustrations represent only a few of the ethanol method comparisons reported, they are consistent with the data presented here in evaluating the REA method with a reference gas chromatographic method.

There are several other points of interest to potential users of the REA ethanol assay which resulted from this study. For example, Abbott recommends when more than twelve samples are run on a carousel that controls be placed after the last sample on the carousel [*2*]. Since the assay is performed at 34°C, there is the possibility of evaporation of ethanol in the later

samples. However, the low, medium, and high controls were run in Positions 1 to 3 and 18 to 20 with each full carousel and no significant decreases in ethanol concentration were noted in controls occupying Positions 18 to 20 when compared with those in Positions 1 to 3.

One concern that was present throughout the study was the possibility of decomposed or badly clotted blood clogging up the TD_x probe. As a precaution, any sample that could not be introduced into an Oxford® or Eppendorf® pipet tip was rejected. Throughout this study, only three specimens were rejected because of this criterion. Numerous samples which were decomposed, clotted, or contaminated with particulate matter were analyzed, and there were no significant differences in analyzing these specimens as opposed to fresh specimens.

In addition to the small sample size requirement, there were other advantages to using the REA method. The assay calibration curve is stable for at least two weeks. In fact, calibrations were performed only when a new lot of reagents was used. No sample pretreatment or ethanol separation steps are required. A carousel of twenty specimens or controls can be analyzed in 20 to 25 min, making the REA ethanol assay a faster analytical method than GC.

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