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Delayed Ethanol Analysis of Breath Specimens: Long-Term Field Experience with Commercial Silica Gel Tubes and Breathalyzer Collection

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ABSTRACT: Delayed ethanol analysis was performed on breath specimens collected with commercial silica gel tubes using multiple Breathalyzer® instruments. Eleven hundred and nine results were obtained from an ethanol testing program over a five-year period. Only 2.5% of the specimens had apparent collection errors. For the valid specimens, the most frequent result was 0.11 g/210 L and the mean result was 0.14 g/210 L. For 642 specimens, delayed results were compared with direct results. Direct results were greater than delayed results for 55%, less than for 27%, and equal to for 18% of the pairs. When fixed tolerance limits of ±0.03 were used, 81% of the direct results were confirmed. The confirmation percentage was best in the critical range of direct results, 0.05 to 0.15 g/210 L. The collection tubes showed no substantial variability in retaining ethanol during storage and releasing ethanol for analysis.

KEYWORDS: toxicology, ethanol, breath-alcohol testing devices

The use of breath as a specimen for analysis has long been attractive, especially in those situations in which medical personnel are not present to perform phlebotomy. One such situation is the procedure of evidentiary ethanol testing by law enforcement personnel at the time a suspect is detained. Breath-ethanol analysis has been used in connection with traffic law enforcement in several states. This approach has been criticized when results were used to derive a corresponding blood-ethanol concentration, because of variability in the partitioning for ethanol between blood and breath. However, this problem is resolved when, according to the recommendation of Mason and Dubowski [*1,2*], results are reported in units of a breath concentration, and legal statutes define limits in terms of a breath concentration. For example, in the state of Oklahoma, statutes define the collection, analysis, and interpretation of results related to driving while impaired or intoxicated in terms of breath specimen concentrations at the time of the test.

It has been debated whether breath specimens should be retained for confirmatory analysis at a later time. In a few states, including Oklahoma, a second breath specimen,

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the "retained" breath-alcohol specimen, is collected at the time of "direct" breath-alcohol analysis and held for subsequent analysis. Regulations also define the maximal difference between the two results for confirmation.

In order to fulfill the requirement for specimen retention, a trapping material must be available for capturing and retaining ethanol and releasing it at the time of analysis. Silica gel and magnesium perchlorate have been frequently used for this purpose. Silica gel, along with Breathalyzer® instrumentation, has been recommended [*3*], but no long-term field experience with this combination has been reported. We report here a retrospective study using such a combination, as employed in a state breath-ethanol testing program.

Materials and Methods

Specimens

Each retained breath-ethanol specimen was collected in the field following a defined protocol with a Breathalyzer 900/900A instrument (Smith and Wesson Co. Pittsburgh, Pennsylvania) by trapping a 52.5-mL sample of mostly alveolar air onto silica gel contained in a collection tube (ToxTrap, Inc., Dover, Delaware). During the period of study, specimens were collected at a variety of field locations, by numerous law enforcement personnel, using multiple lot numbers of collection tubes, and with several Breathalyzer instruments. (Approximately 225 instruments were in field use.) Specimens, along with chain-of-custody documentation, were forwarded to our Forensic Alcohol Laboratory at the Oklahoma Medical Center, Oklahoma City, for delayed ethanol analysis. Specimens were stored at ambient laboratory temperature until the time of analysis. Between June 1984 and July 1989, a total of 1109 delayed ethanol measurements were performed.

Analytical Method

Retained breath-ethanol specimens were analyzed by direct-injection gas chromatography [*4*]. Accordingly, the silica gel from each collection tube was carefully transferred to a vial and 1.0 mL of deionized water was added. The vial was tightly sealed and allowed to equilibrate for 1 h, with periodic shaking at 15-min intervals. To 0.20 mL of the aqueous solution, 0.10 mL of internal standard, *n*-propanol (30 μL/L volume/volume), was added. An aliquot was injected directly into a gas chromatograph equipped with a 6-ft (1.8-m) by 4-mm (0.16-in.) glass coiled column packed with GP 60/80 Carbopack B/5% Carbowax 20M and a flame ionization detector. The retention, relative to the internal standard, for methanol, acetone, ethanol, and 2-propanol was 0.23, 0.34, 0.42, and 0.64, respectively. Five aqueous ethanol solutions, ranging from 8 to 96 mg/L, were analyzed with each batch of specimens and used to calculate the ethanol concentration of the unknowns. A factor was used to convert the results to a breath-ethanol concentration. The factor was derived by analyzing various lot numbers of ToxTrap collection tubes prepared with known quantities of ethanol and was verified with similar material each time the assay was performed. The final results, expressed as grams of ethanol per 210 L of breath, were truncated to the second decimal place. Precision for the chromatographic method at an ethanol concentration of 0.10 g/210 L breath was 3.7 and 4.3% for within-run and between-run coefficients of variation, respectively.

Results

Over a five-year period, a total of 1109 breath-ethanol analyses were performed in our Forensic Alcohol Laboratory. The most frequently occurring result was 0.11 g/210 L (102/1109 or 9%). Twenty-seven results were at the lower limit of detection (<0.02 g/

210 L), which would be consistent with breath collection errors. The greatest measured concentration was 0.75 g/210 L, which was more than twice the concentration of any other specimen; it is likely that this value also resulted from a collection problem. Therefore, for 28 specimens (2.5%), improper collection of a breath sample on the silica gel was suspected. After these results were eliminated from the total, the mean result for the remaining 1081 results was 0.14 g/210 L. The distribution of the 1081 results is shown in Fig. 1.

To evaluate the procedures for delayed ethanol testing, results from our laboratory were compared with direct results measured using Breathalyzer instruments in the field. For 642 results, corresponding Breathalyzer results were obtained. The distribution of these delayed breath-ethanol results, which was very similar to that of the total delayed results, is also shown in Fig. 1. The mean result for the 642 delayed measurements was 0.13 g/210 L, compared with 0.14 g/210 L as the mean of all results. The distributions of delayed and direct results are shown in Fig. 2. The mean of the direct results was 0.14 g/210 L. Regression analysis was used to estimate the relationship between the two measurements. Although both measurements were obtained with imprecision, the direct result was defined as the independent variable. The regression equation for the 642 pairs was $y = 0.839x + 0.013$ with a correlation coefficient of 0.82 and a standard error of the estimate of 0.028. The 80% confidence interval was ± 0.03 g/210 L from the regression line. The distribution of the differences between the direct and delayed results is shown in Fig. 3. The direct result was greater than the delayed result for 55%, less than for 27%, and equal to for 18% of the pairs. For the purpose of confirmation, according to Oklahoma state regulations, "results of analyses of retained breath-alcohol specimens which are within three-hundredths (0.03) g/210 L of the results of the corresponding direct breath-alcohol analysis performed on the same subject shall be deemed confirmatory and substantiative of such direct breath-alcohol analysis results, as a scientifically acceptable tolerance" [5]. For the 642 pairs, 520 (81%) had a difference between −0.03 and +0.03. At the critical value of 0.10 g/210 L, 66 of 75 (88%) of direct results were thus confirmed. Figure 4 shows the percentage of direct results confirmed for various ranges of direct results when fixed tolerance limits of ±0.03 are used. The confirmatory rate was best for direct results of 0.05 to 0.15 g/210 L and substantially decreased for larger results.

Since the delayed result was less than the direct result for a majority of pairs, the question of stability of ethanol with storage on silica gel arose. To investigate this possibility, the number of days between specimen collection and analysis, that is, the number of storage days for the retained specimens, was determined. The average number of storage days for these specimens was 47 days. For those specimens in which the delayed result was less than the direct result, the average number of storage days was 46; for those specimens in which the delayed result was greater than the direct result, the average number of storage days was 48.

To evaluate the recovery of ethanol from the collection tubes, 34 ToxTraps, containing various lot numbers of silica gel, were charged using an ethanol simulator solution containing 25 μg ethanol/52.5 mL of vapor (equivalent to 0.1 g/210 L breath) and analyzed by the delayed breath-ethanol procedure. The average recovery of ethanol was 20.5 μg (82%), with a coefficient of variation of 4%.

Discussion

This retrospective study, using data from the routine operation of a breath-ethanol testing program, reports the performance from a long-term field situation. Recently, this subject was addressed using other combinations of equipment in field studies [6–8], but

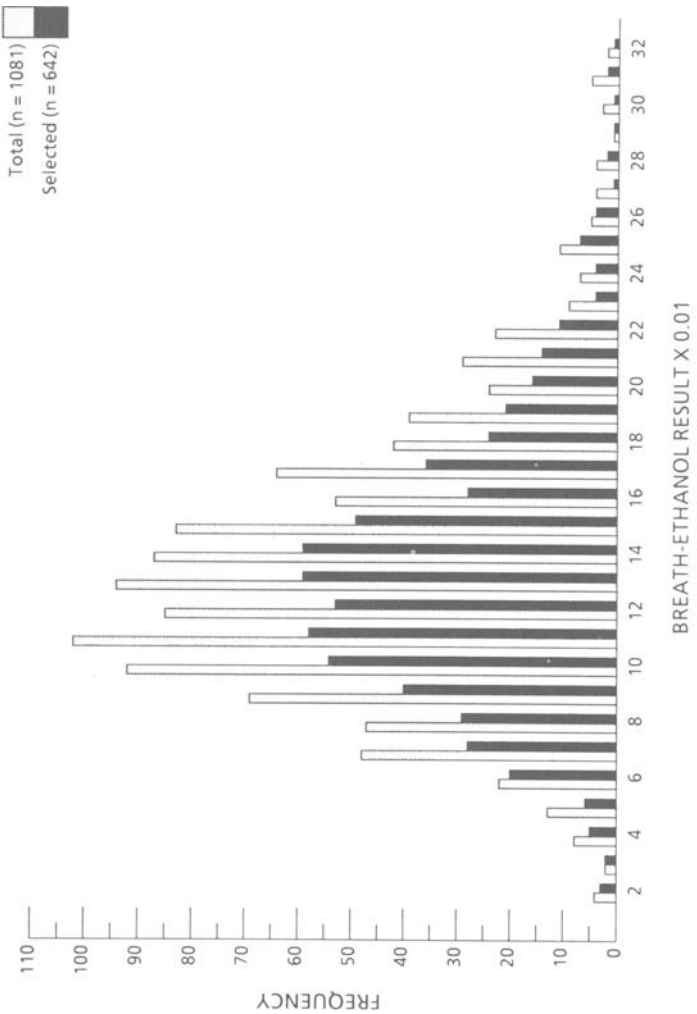

FIG. 1—*Distribution of delayed breath-ethanol results from samples collected on silica gel via Breathalyzer® instruments and measured by gas chromatography. [The total* (n = *1081) represents all valid results; the selected results* (n = *642) represent results for which the corresponding direct result could be obtained.]*

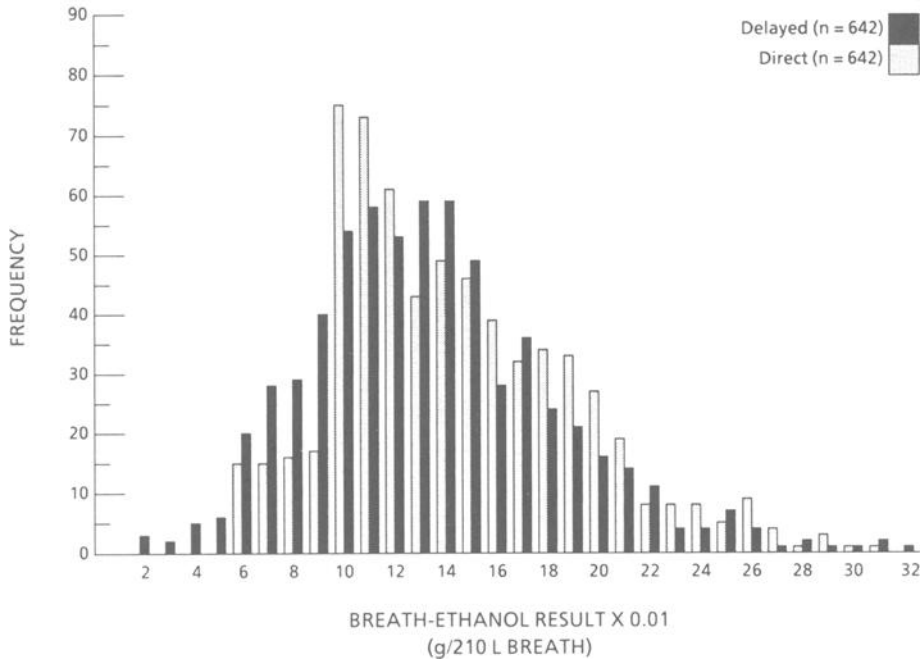

FIG. 2—*Distributions of delayed and direct breath-ethanol results.*

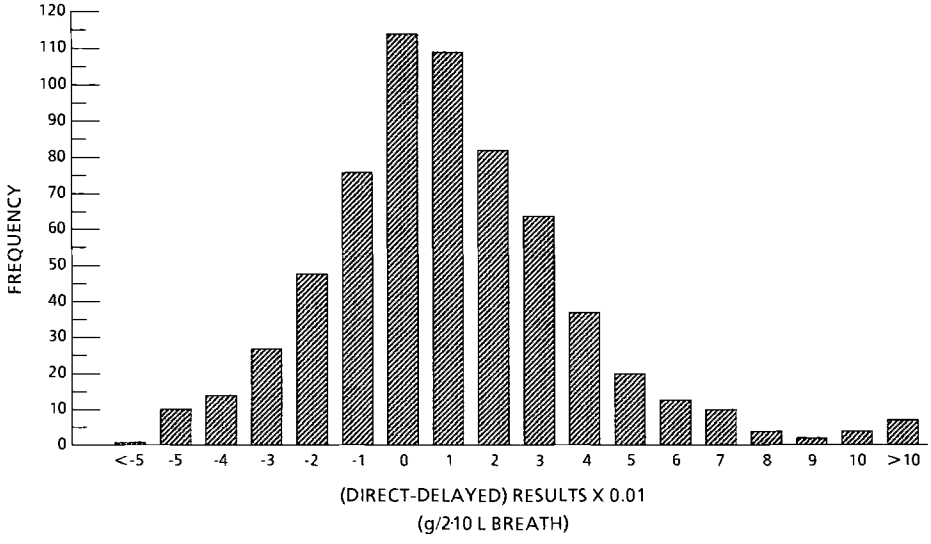

FIG. 3—*Distribution of the differences between direct breath-ethanol results and delayed breath-ethanol results for 642 pairs.*

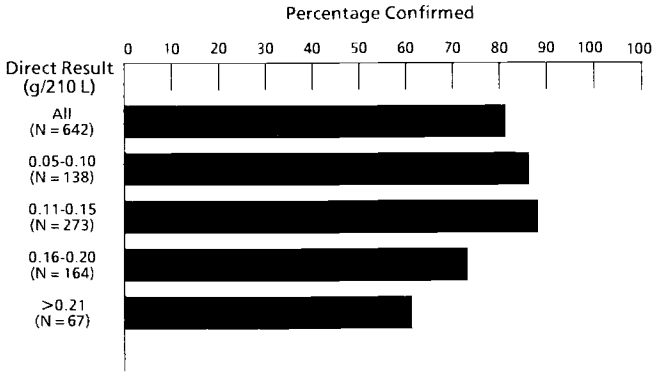

FIG. 4—*Percentage of direct breath-ethanol results confirmed by delayed breath-ethanol results within the ±0.03 g/210 L tolerance limits.*

experience with silica gel as a sorption material and Breathalyzer instruments as collecting devices has not been previously reported.

For delayed breath-ethanol testing to be performed, a sorption material must be used to capture ethanol, to retain it during storage, and to release it at the time of analysis. Silica gel and magnesium perchlorate have been used most often as the sorption material. Goldberger and Caplan [*3*], based on their laboratory evaluations, have concluded that silica gel is the preferred technique. Although the Breathalyzer does not allow the retention of the breath sample used in the direct ethanol measurement, it can be used to collect a second breath sample for delayed alcohol analysis. We identified 28 specimens out of 1109 in which collection errors were apparently made. Most of these (27/28) probably occurred when the individual collecting the breath failed to uncap the collection tube and thus allow the breath to pass over the silica gel. As a consequence, no detectable ethanol was present. In the remaining specimen with a delayed breath-ethanol result of 0.75 g/210 L, the collection tube may have been filled with multiple breaths. Therefore, 2.5% of the specimens were improperly collected. Harding and Field [*9*] reported a similar percentage of poor specimens when the Breathalyzer was used for direct ethanol analysis. Considering the number of personnel, Breathalyzers, and lot numbers of silica gel involved in the collection process, it appears that the percentage of apparently improperly collected specimens in our experience is quite small.

Retained breath-ethanol specimens were stored in a variety of locations for variable time periods. Delayed analysis was performed on average 47 days after collection. It did not appear that the differences between direct and delayed results were dependent on the length of storage; that is, those specimens which showed a smaller difference were not stored for a substantially shorter length of time.

Dubowski and Essary [*10*] reported that release of ethanol from silica gel is incomplete. Using multiple lot numbers of collection tubes, we determined that the average recovery of ethanol from the silica gel was 82%. The delayed ethanol results in this report were corrected for incomplete recovery using silica gel traps. The "factor" used in the analytical method to convert results to a breath-ethanol concentration also corrected for incomplete recovery. The large percentage of results which were confirmatory of direct results further suggests that this procedure of calibration is appropriate. Although the release of ethanol is incomplete, it appears to be consistent within the tolerance limits set for confirmation.

Overall, the performance of the commercial collection tubes containing silica gel and the Breathalyzer as a collection device permitted the confirmation of most (81%) of the direct results. The confirmation percentage was best in the critical range of direct results,

0.05 to 0.15 g/210 L, where alcohol-related offenses are legally defined. When fixed tolerance limits were used throughout the range of values, as defined by law in the state of Oklahoma, the confirmation rate decreased with larger direct results. When the direct result was not confirmed by delayed analysis, even when both results exceeded 0.1 g/210 L, it was not possible to state reliably which result was correct, because there were potential errors which could contribute to inaccuracies in both results.

This paper describes the field performance of a commercial breath-collection device containing silica gel for delayed ethanol testing. Although the application of this methodology has been primarily in the area of traffic law enforcement, the technology could be employed in other situations. For example, collection could be performed by this noninvasive technique at the workplace and analysis performed later at a remote site.

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