PROFESSIONAL

Repeatability of replicate breath alcohol measurements collected in short time intervals

RG GULLBERG

Washington State Patrol, Breath Test Section, 811 East Roanoke, Seattle, WA 98102, USA

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The effect of short time interval sampling between replicate breath alcohol samples has been investigated. The results from 10 samples, which were collected approximately one minute apart from eight individuals and approximately 20 seconds apart from one individual, were evaluated by simple linear regression. The regression coefficient (slope) and its standard error were evaluated for the presence of any trend in alcohol depletion. Other statistical analyses were also included in this assessment. All nine subjects had linear regression coefficients for the end-expiratory results that were not significantly different from zero ($P > 0.05$). In view of the respiratory physiology, there does not appear to be any measurable depletion of breath alcohol concentration due to sampling intervals as short as one minute.

Untersuchung zum Einfluss von Kurzintervallmessungen bei repetitiver Atem-Alkohol-Bestimmung. Die Ergebnisse von je 10 Proben, erhoben in Abständen von 1 Minute bei 8 verschiedenen Personen, wie auch Proben erhoben in Abständen von 20 Sekunden bei der gleichen Person, wurden mittels einfacher Linearregression ausgewertet. Der Regressionskoeffizient (Neigung) und seine Standardabweichung wurden für erkennbare Trends im Alkohol-Abbau berechnet. Alle Testpersonen zeigten bei den Endausatmungsergebnissen Linearregressionskoeffizienten, welche nicht sinifikant von Null abweichen ($P > 0.05$). Aus Sicht der Atmungsphysiologie sind scheinbar keine messbaren Fehler in der Atemluft-Alkoholkonzentration vorhanden, auch wenn Messungen in kurzen Intervallen von 1 Minute erfolgen.

L'effet de la prise d'échantillon à des intervalles très courts pour l'analyse d'alcool dans l'haleine a été étudié. Les résultats à partir de 10 échantillons récoltés approximativement à une minute d'intervalle de 8 individus et à 20 secondes d'intervalle pour un individu ont été évalués par régression linéaire simple. Le coefficient de régression (la pente) et son erreur standard ont été mesurées pour déterminer toute tendance de diminution d'alcool. D'autres analyses statistiques ont également été prévues dans l'étude. Les 9 sujets avaient des coefficients de régression linéaire pour les résultats de fin d'expiration qui n'étaient pas significativement différents de zéro ($P > 0.05$). Au vu de la physiologie respiratoire, il n'apparaît pas qu'il y ait de diminution mesurable d'alcool dans l'haleine à cause d'intervalles d'échantillonnage aussi petits qu'une minute.

Se investiga el efecto de intervalos de tiempo cortos en la toma de muestras de alcohol en el aire expirado. Se evalúan mediante regresión linear simple los resultados de 10 muestras que fueron tomadas con un intervalo de un minuto aproximadamente entre ocho individuos y veinte segundos de otro individuo. Se evaluaron el coeficiente de regresión (pendiente) y su error standard en relación con un posible sesgo en la deplección. En esta valoración se incluyeron otros análisis estadísticos. Los nueve sujetos presentaron resultados de la fase expiratoria final no significativamente distintos de cero ($P > 0.05$). A la vista de la fisiologia respiratoria no parece existir ninguna deplección mensurable en la concentració de alcohol, que pueda ser debida a un muestreo en intervalos tan bajos como un minuto.

Key Words: Alcohol; Breath alcohol concentration; Simple linear regression; Replicate analyses.

Introduction

The measurement of breath alcohol concentration (BrAC) is dynamic and complex, involving many biological and analytical considerations. Associated with forensic applications is the heavy burden of ensuring competent and reliable results. Precise results are one method by which confidence is established, with many jurisdictions employing a duplicate breath sample protocol for evidentiary purposes.

In an effort to help jurisdictions establish meaningful and competent testing protocols, the National Safety Council Committee on Alcohol and Other Drugs has published the statement "... the breath samples should be collected at intervals of not less than 2 nor more than 10 minutes, ... results 0·02 g/210 l ... shall be deemed to be in acceptable agreement" [1]. This prudent and appropriate recommendation is likely to have been motivated by the concern for "mouth alcohol" and the prevention of bias from alcohol metabolism over time.

Important questions, however, relate to the two minute minimum. Is there some biological reason why the breath sampling interval should not be less than two minutes? Does breath alcohol deplete significantly from the respiratory system with more frequent sampling? The present study attempts to answer these questions through appropriate experimental design and linear regression analysis. The regression coefficient or slope can be used to assess trends in sequentially performed measurements and provide insight into the effect of short-term replicate breath alcohol analyses.

Methods

Eight individuals (four male and four female) each provided ten consecutive breath samples for alcohol determination in a BAC Verifier DataMaster (National Patent Analytical Systems, Mansfield, Ohio) following alcohol consumption. These individuals were part of the same group reported in an earlier communication [2]. The samples were essentially end-expiratory, being accepted and reported when instrumental sampling criteria were met. Following sample acceptance and reporting, the instrument purges its sample chamber automatically in preparation for the next analysis. The instrument is capable of performing five sequential breath analyses before it must be re-initialized for the next set of five. As a result, the ten replicate measurements ranged from total times of 10 to 12 minutes for the eight individuals. These times were approximate since the instrument records time truncated to minutes.

In addition, one individual (subject 9) was instructed to exhale and inhale continuously through a DataMaster instrument set up to allow for this procedure. A data acquisition system was attached to the instrument detector board to collect the continuous real time data. To represent extremely small sampling intervals, 10 samples ranging from 6·5 to 13·5 seconds of exhalation were collected within 3·7 minutes.

Descriptive statistics for each individual were computed along with the F_{max} statistic for between-subject homogeneity of variance. Regression analysis was also performed where each measurement was regressed upon the measurement sequence in order to evaluate the presence of a sequence dependent trend. The linear regression coefficient (slope) along with its 95% confidence interval were thus determined. In addition, an F statistic ($H_o: b_1 = 0$) was computed for each individual's data to determine if the slope differed significantly from zero. Statistical analyses were carried out using SPSS/PC + V3.0 (SPSS, Inc., Chicago).

Regressing measurement results on test sequence also produced residuals which were subjected to an analysis of runs. The procedure evaluates the residual's signs (+ or −) and tests for runs employing a t-statistic looking for deviations from linearity [3]. A non-random sequence of signs will result in too few or too many runs as compared to a random sample [4]. Residual analysis is useful in validating assumptions associated with simple linear regression.

TABLE 1 Statistical summary of repeat analyses ($n = 10$) of end-expiratory breath alcohol concentration by subject.

Subject	*Sex*	*Time (min)*	*Mean (g/210 l)*	*sd (g/210 l)*	*%CV*
1	M	11	0·1107	0·0083	7·5
2	F	11	0·1130	0·0033	2·9
3	F	11	0·0765	0·0019	2·5
4	M	12	0·1202	0·0048	4·0
5	F	11	0·1083	0·0025	2·3
6	F	10	0·0687	0·0023	3·3
7	M	11	0·1087	0·0074	6·8
8	M	11	0·0851	0·0038	4·5
9	M	3·7	0·1191	0·0026	2·2

Results

Table 1, including forensically relevant values, shows that precision varied widely between individuals. The F_{max} test for homogeneity of variances was applied to

the between-subject data and resulted in $F_{max} = 19{\cdot}1$ $P < 0.01$, indicating significant differences in between-subject variability.

Figure 1 shows individual replicate measurements plotted against test sequence for subjects 1 and 9. The best fit regression line is shown along with statistical analyses. The standard error of the estimate (SEE) is a more reliable assessment of fit than is the correlation coefficient (r), since r is influenced by the slope [5]. The SEE is simply the standard deviation of the residuals about the line.

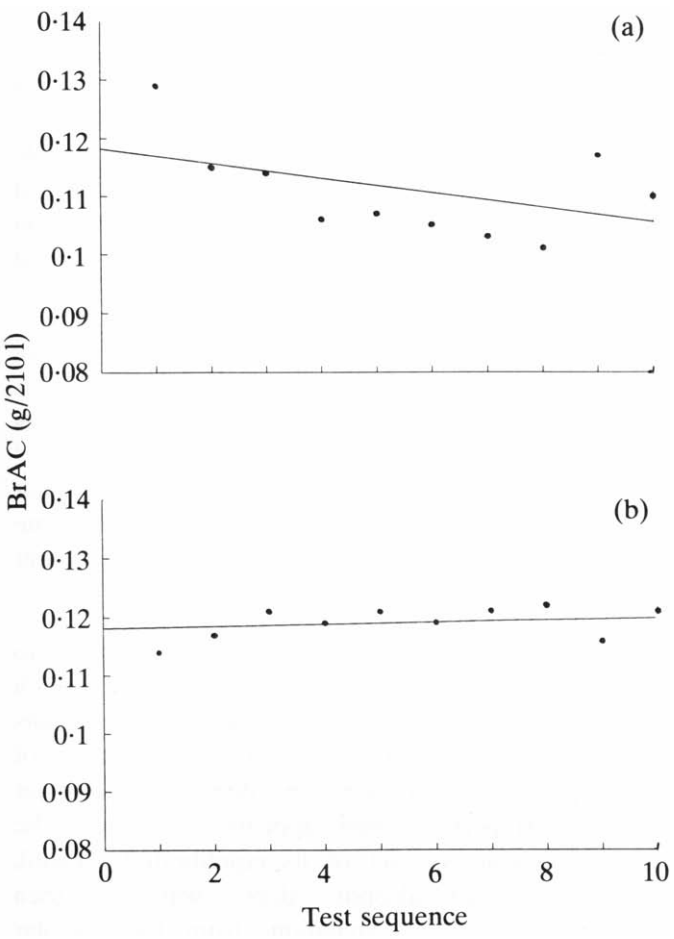

FIGURE 1 Regression line showing the variation of breath alcohol concentration with test sequence. (a) Subject 1 (SEE = 0·0076, BrAC = −0·00141T + 0·118); (b) Subject 9 (SEE = 0·0025, BrAC = 0·00039T + 0·117).

Table 2 shows the results of regression analysis performed on the replicate measurements of each individual. The regression slope (along with its standard error) was the parameter of interest for evaluating trend, and in every case the 95% confidence interval included zero with a non-significant F statistic ($p \geq 0{\cdot}08$). Computing the confidence interval for the slope or regression coefficient is an important aspect of simple linear regression and in some cases is preferred over inferential analysis [6].

Table 3 evaluates the runs for each individual by residual analyses, and notes whether the P value was significant at the 5% level. Significant P values indicate a non-random distribution of runs and thus non-linearity in the sequential measurements. Since this is a 2-tailed test, either too many runs or too few runs could have resulted in statistical significance. Between four and nine runs would be considered non-significant at the 5% level with nine degrees of freedom. Two subjects produced significant t values ($P < 0.05$), resulting from too few runs to be described as random, thus suggesting non-linearity.

Discussion

The present results are limited in both number of subjects and magnitude of breath alcohol concentrations, but the approach is relevant from both its design and forensic perspectives.

It would be unusual for a jurisdiction to allow only one minute between breath alcohol measurements, or to require ten measurements (two or perhaps three being more typical). Therefore, the present study represents extreme conditions by design.

The slope is the parameter of interest in the present context since it is a means of assessing any significant change in BrAC over the total sampling interval. The slope is an appropriate summary statistic to use in evaluating change in repeated measurement data [7]. The application of simple linear regression in this context appears to be justified since the independent variable (test sequence) is without error, thus complying with an important assumption [8]. Only two of the subjects showed possible non-linearity in residual analysis where their number of runs was three. The residual analysis, therefore, supports another important assumption in simple linear regression—a uniform distribution of residuals about the line. Thus, the near zero slope supports the independence of BrAC with test sequence. Even to the extreme of sequential respiratory cycles, frequent sampling does not appear to deplete the respiratory system of ethanol.

The significant differences between individual variability (F_{max} test) can be due to factors such as non-uniformity of exhalation, breathing pattern prior

TABLE 2 Regression analysis of end-expiratory breath samples ($n = 10$) plotted against test sequence for 9 subjects.

Subject	*Slope* (*g*/210 *l*)	*SE of slope* (*g*/210 *l*)	95% *confidence interval* (*g*/210 *l*)	*F test*
1	−0·00141	0·00084	−0·0033 to 0·00054	2·85 ($p = 0·130$)
2	0·00044	0·00036	−0·00039 to 0·0013	1·49 ($p = 0·257$)
3	0·00027	0·00020	−0·00019 to 0·00073	1·86 ($p = 0·210$)
4	0·00072	0·00051	−0·00046 to 0·0019	2·00 ($p = 0·196$)
5	−0·00032	0·00027	−0·00094 to 0·00030	1·37 ($p = 0·275$)
6	−0·00030	0·00024	−0·00085 to 0·00025	1·50 ($p = 0·256$)
7	0·00024	0·00086	−0·0017 to 0·0022	0·075 ($p = 0·791$)
8	0·00073	0·00037	−0·0016 to 0·00012	4·01 ($p = 0·080$)
9	0·00039	0·00028	−0·00026 to 0·00104	2·04 ($p = 0·191$)

to exhalation, instrumental differences, etc. The test does not really address the issue of trends in replicate analyses but does support the intuitive notion of biological variability.

TABLE 3 Residual analysis showing the evaluation of runs.

Subject	*No of runs*	*t*
1	3	−2·76*
2	5	−1·10
3	3	−2·76*
4	5	−1·10
5	6	−0·27
6	5	−1·10
7	6	−0·27
8	6	−0·27
9	6	−0·27

* $p < 0·05$.

The human respiratory system is dynamic and complex. The air/blood interface in the alveoli is predominantly (though not exclusively) where gas exchange occurs, with ethanol partitioning between air and blood as a function of temperature and blood water (Henry's Law). Human lungs contain approximately 300 million alveoli, a surface area of approximately 70 m^2, and a membrane/interstitial fluid complex approximately 0·1 micron thick (in the healthy state) that separates blood from air, resulting in a large surface/thickness ratio [9, 10]. The pulmonary blood flow is approximately 90 ml/sec. [11]. This very efficient design provides the anatomical basis for the dynamics of gas exchange. The alveolar region is also highly perfused by capillary blood, resulting in a low ventilation–perfusion ratio. Ethanol partitions itself across the air/blood alveolar membrane virtually unhindered by the membrane itself. By contrast, the upper respiratory tract or dead-air-space region has a low surface/thickness ratio and a high ventilation/perfusion ratio. The airways and upper respiratory tract are also sites of considerable interaction with ethanol due largely to water and temperature factors. This gives rise to concentration heterogeneity throughout the respiratory system and explains much of replicate measurement variability.

The combination of respiratory dynamics and ethanol properties provides for rapid equilibration. It is estimated that ethanol partitions itself and achieves equilibrium in the alveolar region in 10^{-5} to 10^{-4} seconds [11, 12]. With such rapid equilibration it is not surprising that multiple breath samples in a short time interval do not significantly alter measurement repeatability.

It has been shown that hyperventilation prior to exhalation can result in significantly reduced breath alcohol measurements [13]. However these results have more to do with the dynamics and anatomy of the upper respiratory tract than a depletion of alveolar ethanol. Hyperventilation appears to deplete the upper respiratory tract of its equilibrated ethanol, resulting in an alcohol "debt" which is then replenished, during exhalation, from the alveolar breath [14]. Hypoventilation or rebreathing, on the other hand, appears to have the opposite effect by establishing a more uniform and homogeneous breath alcohol concentration throughout [14]. An individual typically provides approximately 12 exhalation cycles of tidal volume air (approximately 500 ml) per minute [11] which should be adequate to restore respiratory equilibrium in regards to breath alcohol concentration.

Finally, it is important to note that allowing at least two minutes between breath samples for evidential

purposes will help to address the issue of "mouth alcohol bias". Two minutes is generally adequate to allow the measurements to be significantly different if "mouth alcohol" is biasing one of the results. This is due to the first-order or exponential elimination of "mouth alcohol". Therefore, it is not advised that jurisdictions perform duplicate analyses one minute apart. However, extremely short sampling intervals do not appear to influence replicate measurement variability significantly, and should not be the basis for rejection.

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