Examination of Some Performance Characteristics of Breath Alcohol Measurements Obtained with the Intoxilyzer® 8000C Following Social Drinking Conditions

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Abstract

The Intoxilyzer 8000C was used to measure breath alcohol concentration (BrAC) in 10 healthy subjects under social drinking conditions. Measurements commenced within 5 min of the end of drinking (EOD). For 14 blood-breath pairs, measured BrACs were compared to corresponding venous blood alcohol concentrations (vBAC) of samples drawn at least 30 min after EOD and within 5 min of the corresponding breath test. BAC was analyzed using an enzymatic method. Concentration differences between breath and blood (BrAC – vBAC) ranged from –32 to +3 mg/dL (untruncated BrAC) and from –32 to –4 mg/dL (truncated BrAC). The Invalid Sample message was actuated in five out of 23 BrAC profiles. In the remaining 18 samples, residual mouth alcohol was evaluated by comparing the maximum difference between successive (5 min apart) measurements (MID5) over 20–30 min after EOD with the precision of replicate BrAC values taken 30–40 min after EOD (5 mg/dL or less; precision unaffected by breath sample volume over the range of 2–3 L). MID5 values occurred within the first three measurements in 16/18 cases, indicative of a significant mouth alcohol effect. Thus, mandatory delays should be used with the Intoxilyzer 8000C prior to testing to minimize the probability of overestimation of BrAC due to mouth alcohol.

Introduction

Characterization of the performance indicators of breath alcohol analyzers is critical to their utility in the prosecution of offenses related to drinking and driving. The Criminal Code of Canada declares that it is an offence to operate or to have care or control of a motor vehicle with a blood alcohol concentration (BAC) in excess of 80 mg/dL. Accordingly, the breath of suspected drinking drivers is often analyzed in order to ascertain whether this BAC limit is exceeded. Given that the penalty associated with conviction of such offences is severe, the accuracy and precision of breath alcohol measurements requires thorough characterization for each instrument used in the field.

Various reports have described the precision of breath alcohol analyzers using in vitro (1–3) and in vivo (4,5) measurements. In practice, a large component of variability in breath alcohol concentration (BrAC) measurements is the result of variability in the breath sample itself (i.e., biological variability) (3). Consequently, any assessment of instrument precision must include in vivo breath sampling. Indeed, a given jurisdiction may have defined criteria in terms of minimally acceptable criteria for the precision of replicate breath tests (6).

The accuracy of breath alcohol instrumentation has been described with respect to correlation of a BrAC value with a corresponding BAC (7–12). Often these reports make use of data collected in the field using subjects assessed as part of investigations of suspected alcohol-related offences, where an evidentiary breath test result is compared to a BAC measurement from a sample taken as part of the investigation (7–9,12). Because blood sampling often occurs some time after breath sampling in these cases, analytical results are usually corrected for the ethanol metabolized between the collection of the different sample types. Although this approach has merit in describing the instrumentation in actual field use, it may be somewhat skewed by the use of a single β-phase elimination rate [e.g., 19 mg/dL/h (13)]. Conversely, laboratory studies may facilitate the use of simultaneous blood and breath sampling; however, care should be taken to avoid highly controlled dosing conditions where subjects are provided with a large, bolus dose of ethanol to be consumed over a short time period as is routinely done for studies of ethanol pharmacokinetics (14).

The Intoxilyzer 8000C is a relatively new breath alcohol analyzer, recently approved by the Canadian government for evidentiary use in assessment of suspected drinking drivers. Ac-

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cordingly, characterization of the accuracy and precision of the Intoxilyzer 8000C is critical. Here, we report the use of the Intoxilyzer 8000C in a laboratory study involving the measurement of breath alcohol profiles in healthy volunteers following social drinking conditions. By collecting breath measurements shortly after drinking ceased with short inter-measurement intervals, we examined the influence of residual mouth ethanol on BrAC measurements and the precision of replicate measurements with minimal influence from metabolic effects. Further, venous blood samples were drawn at least 30 min after the cessation of drinking, so that venous blood alcohol concentration (vBAC) and BrAC could be compared in an assessment of instrument accuracy under social drinking conditions.

Methods

Alcohol consumption

Healthy volunteers (n = 10, n_{male} = 5), ranging in age from 19 to 27 years, consumed ethanol under social drinking conditions (~ 2–4 standard drinks over the period of roughly 1 h), following the provision of informed consent. All subjects were verified to be of legal drinking age in Canada (19 years). The dose administered was 0.6–0.7 g/kg. In order to minimize experimental constraints that may otherwise reduce forensic applicability, subjects were permitted to choose their beverage and were not instructed to abstain from eating prior to arrival. Subjects chatted freely during the drinking and breath testing process, and light snacks (e.g., potato chips, popcorn, etc.) were permitted after blood sampling when desired. Two subjects were examined in any given drinking session. All subjects completed at least two separate drinking sessions three weeks apart, each time consuming the same dose and beverage. One subject completed three sessions, while another subjected completed four sessions. The study protocol was approved by the Laurentian University Research Ethics Board. The details describing the subjects and the doses of ethanol consumed are summarized in Table I. All subjects were monitored continuously in terms of BrAC and remained onsite until a final BrAC reading between 10 and 20 mg/dL (BAC equivalent, using a 2100:1 blood/breath ratio) was observed.

Table I. Summary of Subject Characteristics and Beverages Consumed

Subject*	Age	Body Weight (kg)	Beverage Consumed (Alcohol Content, v/v)	Total Ethanol Dose (g/kg)†
M1	27	83	Beer (5%)	0.59
M2	21	61	Beer (5%)	0.59
M3	21	75	Rum (40%) diluted in cola	0.59
M4	21	107	Rye whiskey (40%) diluted in cola	0.69
M5	19	75	Beer (5%)	0.69
F1	22	74	White wine (13%)	0.59
F2	22	61	White wine (13%)	0.59
F3	19	56	Vodka cooler (7%)	0.59
F4	22	61	Sangria (6%)	0.59
F5	24	89	Vodka cooler (5%)	0.59

* Subjects were coded according to gender (i.e., Subject M1 was male subject #1, whereas subject F5 was female subject #5).
† The total ethanol dose was divided into 2–4 drinks, consumed at the subjects own pace, over approximately 1 h.

BrAC measurements

Within 5 min of completion of their dose, subjects provided breath samples into an Intoxilyzer 8000C equipped with a model PS-590 portable wetbath simulator, operated in manual breath sampling mode (i.e., the full evidentiary breath testing sequence was not employed). To verify instrument calibration, the instrument response to standard alcohol solution (SAS, target value of 100 mg/dL) introduced via the simulator was used. Calibration was deemed acceptable when an instrument response of 90–110 mg/dL was achieved over five replicates. Subjects provided breath samples in alternating sequence without any delay into the Intoxilyzer 8000C, resulting in a given subject providing samples with a frequency of approximately 1 sample/5 min over at least 2 h. The breath sample volume provided (as indicated by the sample volume measure on the digital display) was targeted to be approximately 2 L, and all other measures of breath sample suitability (duration, pressure) were verified through the standard instrumental response.

The frequency of incidence of the "Invalid Sample" indicator was noted, and the time course of measured breath alcohol concentration was assessed during the first hour of sample collection in an effort to examine the ability of the instrument to discern the presence of residual ethanol in the mouth that may otherwise elevate the measured BrAC.

Effect of sample volume on precision of replicates

The Intoxilyzer 8000C provides a measure of breath sample volume on the digital output display. To examine the effect of variability of breath sample volume, each subject provided successive breath samples with the instruction to blow until the display reached a particular target volume. Target volumes chosen were 2.0, 2.5, and 3.0 L with 2–5 samples provided at each target volume. As breath samples were collected continuously throughout the session, samples for this part of the study were collected once the subjects were clearly within the declining phase of the blood alcohol curve and providing BrAC measurements that were generally within the range of 30–50 mg/dL.

Analysis of blood alcohol concentration

Venous blood samples were analyzed for ethanol content using an assay based on enzymatic ethanol oxidation with spectrophotometric detection. Venous blood was drawn from the cubital vein into BD Vacutainer® tubes containing 15 mg sodium fluoride and 12 mg sodium oxalate. The blood volume collected was roughly 6 mL or less in each case. Blood alcohol assay kits (Immunalysis, Pomona, CA) were used in conjunction with a ChemWell 2910 Automated EIA and Chemistry Analyzer

(Awareness Technologies, Palm City, FL) in the analysis. Calibrants were prepared as serial dilutions of an aqueous ethanol standard (300 mg/dL, Cerilliant, Round Rock, TX) in deionized water in combination with ethanol-free blood such that 100 µL blood was combined with 100 µL ethanol standard to yield final ethanol concentrations of 0, 10, 25, 50, and 100 mg/dL. Positive controls were prepared similarly to yield a final ethanol concentration of 75 mg/dL. Subject blood samples (100 µL) were mixed with 100 µL deionized water prior to analysis. All samples generated four measurements, with two aliquots drawn from a given sample and each aliquot analyzed in duplicate.

All samples were mixed and combined with 400 µL of 0.5 N perchloric acid in polypropylene tubes and vortex mixed for 30 s. Samples were then incubated at room temperature for at least 5 min and then centrifuged (4000 rpm, 5 min). Supernatant (20 µL) was combined with 100 µL assay buffer (Reagent A: 0.6 M Tris Buffer, 0.1% sodium azide preservative) and 100 µL enzyme reagent [Reagent E: alcohol dehydrogenase (ADH) and NAD cofactor in 0.1% sodium azide preservative]. The mixtures were incubated at room temperature for 30 min with the instrument sash drawn to minimize external light exposure. Following the incubation period, the absorbance of the wells at 340 nm was measured.

Table II. Examination of Mouth Alcohol Contributions to Initial BrAC Measurements and Incidence of the Invalid Sample Indicator*

Subject	INVALID SAMPLE Actuated?	Max Incremental Difference (MID, mg/dL)	Corresponding Time (min) Sample #	3 × SD (mg/dL) Replicate Samples
M1-01	YES	17	7/3	6.9
M1-02	NO	61	3/2	13.5
M2-01	NO	42	3/2	9.9
M2-02	NO	14	3/2	8.7
M2-03	NO	37	2/2	7.6
M2-04	NO	26	2/2	6.6
M3-01	YES	12	6/3	3.0
M3-02	NO	42	4/2	12.1
M3-03	NO	127	5/2	3.5
M4-01	NO	10	4/2	15.4
M4-02	NO	1/6†	11/3 [31/5]†	10.4
M5-01	NO	7	4/2	6.7
M5-02	NO	46	5/2	10.8
F1-01	YES	7	15/4	4.0
F1-02	NO	7	4/2	4.9
F2-01	YES	9	7/3	11.3
F2-02	YES	8	15/5	6.9
F3-01	NO	7	3/2	5.6
F3-02	NO	5	21/7	10.9
F4-01	NO	6	4/2	6.5
F4-02	NO	4	5/2	4.6
F5-01	NO	6	4/2	8.1
F5-02	NO	28	5/2	12.5

* The maximum incremental difference between successive breath tests (MID5), time post-drinking and breath sample in which MID occurred are shown, along with the precision of replicate measurements for each subject.
† MID was found at 31 min but within the 20 min window under consideration for MAE. The MID5 was noted at 11 min. In both cases, the MID5 < 3 × SD.

Results

In this work, BrAC values are reported in terms of the blood alcohol concentration equivalent (mg/dL) based on a blood-breath ratio of 2100:1 used to calibrate the Intoxilyzer 8000C, as is currently practiced in the field in Canadian jurisdictions. This is numerically equivalent to reporting BrAC values in units of mg/210 L.

Precision of replicate measurements and examination of mouth alcohol contributions

The data obtained show that of a total of 23 BrAC profiles collected, the "Invalid Sample" indicator was only actuated in five cases. In all of those cases, the "Invalid Sample" indicator only appeared during the first sample. Additionally, the precision of the BrAC measurements was examined through measurement of the standard deviation (mg/dL) in 4–6 measurements. These were collected over a period of ~ 20–30 min beginning 30–40 min after the end of drinking in order to minimize confounding effects of alcohol elimination. These data showed replicate testing precision (SD) to range from 1.0 to 5.1 mg/dL. These data were then used to compare against the maximum incremental difference (MID) in BrAC between successive measurements as a function of time in order to discern if there were clear drops in measured BrAC that may be associated with the mouth alcohol effect (MAE) in the early stages of breath measurement (i.e., 0–20 min). These data are summarized in Table II. The features used in the measured BrAC profiles to determine the replicate test precision and MID are illustrated in Figure 1.

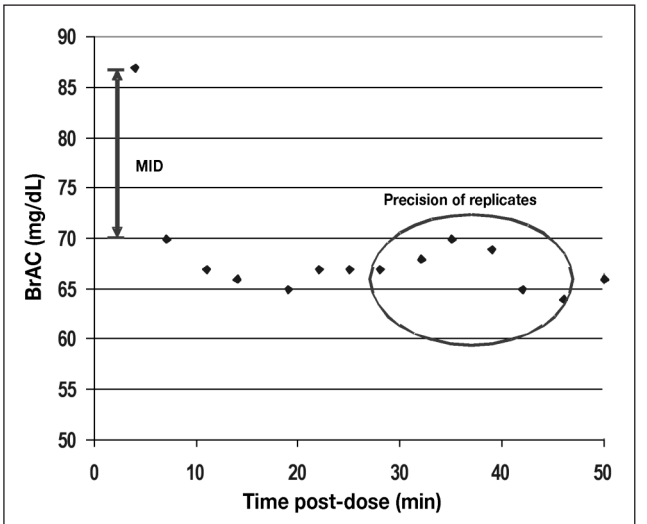

Figure 1. BrAC profile (M1-01) highlighting the parameters used in estimation of effect of residual mouth alcohol. MID5 = Maximum Incremental Difference between successive BrAC measurements taken roughly 5 min apart. The plot highlights the window over which replicate measurements were included for determination of replicate test precision (3 × SD).

Effect of sample volume on precision of replicates

The mean (± SD) and maximum and minimum BrAC measurements for each subject at each target volume are summarized in Table III along with the maximum difference between any two truncated BrAC measurements within the set. Subjects were instructed to observe the digital display of the Intoxilyzer 8000C while providing a sample and continue to blow until the displayed breath volume reached a predefined target (2.0, 2.5, and 3.0 L). Subjects generally provided at least three samples at each target volume, and samples were provided sequentially with target volumes set in the order (2.0, 2.5, and 3.0 L). Typically, subjects were able to provide samples close to those limits (± 0.3 L). The time required for collection of these samples ranged from 12 to 38 min with a mean value of 20 ± 7 min.

Comparison of breath alcohol measurements with BACs measured by the enzymatic method

Analyses of positive control blood samples (75 mg ethanol/dL) yielded results ranging from 70 to 82 mg/dL on five different days. The mean (± SD) positive control result was 77.6 ± 4.6 mg/dL, [coefficient of variation (CV) = 5.9%]. The assay was shown to display good linearity over the concentration range 0–100 mg/dL (R^2 ranged from 0.984 to 0.997, mean: 0.993, SD: 0.006, n = 5). Generally, intrasample precision (SD) ranged from 2 to 10 mg/dL in quadruplicate assays of subject samples. Importantly, this precision was affected by sample preparation (collection of sample aliquots, protein/lipid precipitation, and supernatant isolation) as well as handling and analysis of prepared samples by the instrument. Because measurements were based on spectrophotometric detection, some variability could be introduced by incomplete removal of protein or lipid material. Accordingly, in three cases initial replicate blood analyses (n = 4) displayed precision (SD) greater than 10 mg/dL. In all of these cases, the analysis was repeated, and precision was achieved within the limit described. Overall, the corresponding CV values ranged from 2 to 13% with mean

Table III. Summary of Measured BrAC Values (mg/dL, Expressed as BAC Equivalent, Using 2100:1 Blood/Breath Ratio) with Target Breath Volumes of 2.0 L, 2.5 L, and 3.0 L*

Subject	Mean BrAC (mg/dL) ± SD [Min, Max]	Target Sample Volume (L)	Maximum Difference in Truncated BrAC Values (mg/dL)	Subject	Mean BrAC (mg/dL) ± SD [Min, Max]	Target Sample Volume (L)	Maximum Difference in Truncated BrAC Values (mg/dL)
M1-01	41 ± 3 [38,44]	2.0		M5-02	28 ± 0 [28,28]	2.0	
	38 ± 3 [34,43]	2.5			29 ± 1 [28,30]	2.5	
	35 ± 1 [34,36]	3.0	10		28 ± 1 [27,29]	3.0	10
M1-02	32 ± 1 [31,33]	2.0		F1-02	30 ± 1 [29,30]	2.0	
	34 ± 1 [33,34]	2.5			28 ± 1 [28,29]	2.5	
	34 ± 1 [33,34]	3.0	0		27 ± 1 [27,28]	3.0	10
M2-02	39 ± 1 [38,40]	2.0		F2-02	29 ± 1 [29,30]	2.0	
	40 ± 1 [39,41]	2.5			29 ± 1 [28,30]	2.5	
	38 ± 2 [36,40]	3.0	10		28 ± 0 [28,28]	3.0	10
M2-03	35 ± 3 [33,38]	2.0		F3-01	38 ± 1 [38,39]	2.0	
	32 ± 1 [32,33]	2.5			35 ± 3 [32,38]	2.5	
	31 ± 1 [30,32]	3.0	0		32 ± 2 [30,34]	3.0	0
M3-01	53 ± 1 [53,54]	2.0		F3-02	36 ± 1 [35,36]	2.0	
	56 ± 1 [55,57]	2.5			36 ± 2 [35,38]	2.5	
	58 ± 2 [55,59]	3.0	0		37 ± 1 [36,37]	3.0	0
M3-02	44 ± 1 [43,44]	2.0		F4-01†	45 ± 6 [40,53]	2.0	
	43 ± 1 [43,44]	2.5			38 ± 2 [37,40]	2.5	
	43 ± 1 [43,43]	3.0	0		n/a	3.0	20
M4-01	38 ± 1 [37,38]	2.0		F5-01	29 ± 1 [28,30]	2.0	
	36 ± 2 [34,37]	2.5			29 ± 1 [28,29]	2.5	
	36 ± 1 [35.37]	3.0	0		29 ± 1 [28,30]	3.0	10
M4-02	32 ± 1 [31,33]	2.0		F5-02	31 ± 1 [30,31]	2.0	
	33 ± 1 [32,34]	2.5			30 ± 1 [30,31]	2.5	
	33 ± 1 [32,34]	3.0	0		28 ± 1 [27,29]	3.0	10
M5-01	27 ± 0 [27,27]	2.0					
	25 ± 1 [24,27]	2.5					
	23 ± 1 [22,24]	3.0	0				

* Three samples were collected at each target volume in the order of 2.0, 2.5, and 3.0 L. Total time required for collection of all samples ranged from 12 to 38 min with a mean (± SD) value of 20 ± 7 min.
† Subject F4 was unable to provide a sample with volume of 3.0 L. The maximum volume provided was 2.62 L.

and median values of 9% and 8%, respectively.

Blood alcohol concentrations determined by the enzymatic method are tabulated in Table IV. Also tabulated are corresponding breath alcohol measurements (collected within 5 min of blood sampling) and the differences between corresponding measurements for comparison of sample matrices using non-truncated and truncated BrAC measurements.

Discussion

Precision of replicate measurements and examination of mouth alcohol contributions

Typically, studies of the MAE have been designed such that subjects take a fixed volume of ethanol into their mouths, expectorate, and then provide samples of breath into a breath alcohol analyzer for fixed periods of time thereafter (15). Clearly, this approach is valuable in removing the confounding effect of a positive BAC on the BrAC measurements but provides less insight into the influence of the MAE on BrAC measurements of subjects who have consumed alcohol in social settings, as has been pointed out by Langille and Wigmore (18).

Here we examined the influence of the MAE in two ways. First, we examined the frequency of the Invalid sample message that can serve to indicate the presence of residual mouth alcohol to the operator through examination of the slope of the measured BrAC with respect to time. Significant deviation of the slope beyond the limits defined by the manufacturer, which are based on the shape of a common BrAC profile (i.e., rising and plateau phases), may actuate this warning message. Of the 23 BrAC profiles collected, the Invalid Sample message was actuated in five (22%) instances. In all of those cases, the Invalid Sample message was actuated on the first breath test only. In order to examine whether the MAE was influential in other tests, the incremental difference between successive breath tests was measured for the first 20 min after drinking ceased and compared to the SD of replicate breath tests taken at least 30–40 min after the end of drinking (time we could reasonably expect any MAE influence on BrAC measurements to have completely dissipated) (12). In this way, incremental differences between successive measurements taken roughly 5 min apart that were significantly greater than the precision imply that a significant MAE was present and influencing BrAC measurements; and the magnitude of this influence could be indicated by the maximum incremental difference (MID5). Of those test sessions in which the Invalid Sample message was not actuated, 16 out of 18 sessions saw the highest MID5 occurring with the second or third sample (5 min or less after the first sample was provided). In 12 of these cases, the measured MID5 was > 3 × SD of the replicate BrAC measurements. In five of the 23 sessions where an Invalid Sample message was observed, the first breath test could not be included in the calculations for MID5. Consequently, the true MID5 would likely have occurred after the first sample. The data in Table II show that MID5 values exceeded 3 × SD of the replicate BrAC measurements in four out of the five cases, all of which occurred within 15 min of the end of drinking. In the last instance, the MID5 was less than 3 × SD of the replicate BrAC measurements. Importantly, it should be noted that the maximum incremental difference in BrAC measurements may be dependent on the time between successive tests, breath sample volume, and factors such as whether subjects are free to speak in between tests. Thus, further work is required to examine the effects of these parameters.

Overall, although the Intoxilyzer 8000C is capable of detection of residual ethanol in the mouth by means of the Invalid Sample warning, it is clear that the MAE may still be observed when the warning is not seen. Thus, the practice of a prescribed observation period before breath testing of at least 15 min, as is currently practiced in Canadian and other jurisdictions, remains warranted. This would be especially important if the field portability of the Intoxilyzer 8000C is ever fully exploited as a roadside evidentiary instrument.

Precision of replicate measurements and breath sample volume

The Intoxilyzer 8000C features a breath sample volume measurement for each breath test provided. Given the shape of the breath alcohol concentration profile with respect to breath volume (16), it is well-accepted that end-expired breath samples are required to optimally represent the BAC. Therefore, the degree of intoxication that may be presumed. Accordingly, it is reasonable to expect that the effect of variability in breath sample volume on the validity of replicate breath tests may be questioned in legal proceedings. As a result, we examined the effect of breath sample volume on the precision of replicate breath tests in a manner similar to that described elsewhere (17).

These data show that the maximum difference observed in truncated BrAC measurements was 20 mg/dL. Generally in Canadian jurisdictions, two samples are required, taken at least

Table IV. Venous BAC, Corresponding BrAC, and the Differences Between Corresponding Measurements for Comparison of Sample Matrices*

Subject	Venous BAC (mg/dL)	Corresp. BrAC (mg/dL)	BrAC-vBAC (mg/dL)	trBrAC – vBAC (mg/dL)
F1-01	42	39	–3	–12
F2-01	44	41	–3	–4
F3-01	58	51	–7	–8
F3-02	45	48	+3	–5
F4-01	67	67	0	–7
F4-02	93	82	–11	–13
F5-01	88	72	–16	–18
M1-01	70	57	–13	–20
M1-02	58	56	–2	–8
M2-04	62	58	–4	–12
M3-03	59	53	–6	–9
M4-01	64	66	+2	–4
M4-02	102	70	–32	–32
M5-01	51	40	–11	–11

* Using non-truncated and truncated BrAC measurements (mg/dL, expressed as equivalent BAC using 2100:1 blood/breath ratio).

15 min apart, with results considered to be in good agreement when truncated BrAC values differ by no more than 20 mg/dL (6). Thus, over the breath sample volume range examined, there was no evidence to indicate that variability in the sample volume rendered replicate breath tests unacceptable by these standards. It should be borne in mind that the breath sample volume which corresponds to end-expired breath may be expected to vary from individual to individual (depending on factors including respiratory health, body size, etc.). Further, we conducted our sampling such that target breath sample volumes were set in the sequence of 2.0, 2.5, and 3.0 L. Because ethanol metabolism was occurring simultaneously with breath sampling, any increase in BrAC measurements due to increased sample volume may have been offset by the elimination of ethanol. However, sampling times were relatively short with a mean time to collect all samples of approximately 20 min, which would result in relatively small decreases in BrAC presuming a BAC elimination rate ranging from 10 to 20 mg/dL/h. Overall, this phenomenon warrants further detailed investigation with a substantially larger sample size, randomized target sample volume sequences, fully characterized in terms of respiratory function (forced vital capacity), and body mass index.

Comparison of breath alcohol measurements with BACs measured by the enzymatic method

In this work, the analysis of ethanol in venous blood samples was performed using a commercially available enzymatic assay. Unlike some enzymatic assays, which involve direct sampling and analysis of serum or plasma following centrifugation of whole blood, this assay involved sampling of whole blood (100 µL) followed by dilution with an equal volume of water, and subsequent deproteination using 400 µL 0.5 N perchloric acid. Importantly, all calibrants were prepared using ethanol-free whole blood in combination with aqueous ethanol standards in the same blood/water ratio as was generated in preparing the blood samples of the subjects. All calibrants and subject samples were then subjected to the same deproteination and centrifugation steps, and the supernatants were analyzed in the same fashion. For each batch of subject blood samples analyzed, standard blood samples were prepared, analyzed, and used in the calibration of the analysis of that batch.

Consideration of these factors is critical when interpreting the significance of measurements made using an enzymatic assay. A critical feature here was that whole blood was sampled as opposed to serum or plasma. As a result of the different water contents of serum (or plasma) and whole blood, sampling equal volumes of each matrix type (when derived from the same master blood source) will result in different measured alcohol concentrations, where the serum alcohol concentration will exceed the blood alcohol concentration by an average value of roughly 10–20% (19,20). However, because whole blood was sampled in this work, this effect is largely mitigated. Further, Hodgson and Shajani (19) examined the difference between the analysis of whole blood and deproteinized blood supernatant by an assay based on direct-injection gas chromatography (GC). They reported that the supernatant alcohol concentrations exceeded those in the corresponding whole blood by an average of 5% with values ranging from 4 to 9%. However, in that study, both sample types appear to have been compared against instrument response to calibrators prepared in aqueous solution. The method used here overcomes this bias somewhat by using calibrators prepared in whole blood and subjected to the deproteination step along with the sample. Therefore, it is reasonable to expect that the overestimation of a true BAC by this method would be less than that predicted by the study of Hodgson and Shajani (19).

The data in Table IV show that the difference between BrAC (truncated and non-truncated, expressed as BAC equivalents in mg/dL as defined) and corresponding vBAC measurements (expressed as BrAC – BAC), was less than zero in almost all cases (–32 to +3 mg/dL) for non-truncated BrAC measurements and in all cases (–32 to –4 mg/dL) for truncated BrAC measurements. For truncated BrAC measurements, this difference averaged –12 mg/dL. These data are shown graphically in Figure 2. Thus, these data strongly suggest that BrAC measurements made by the Intoxilyzer 8000C will underestimate the corresponding venous BAC in the majority of cases. In these subjects, the BrAC underestimated the vBAC by an average of 9.8% for non-truncated BrAC measurements and 17.4% for truncated BrAC measurements. Clearly, the likelihood of this underestimation may be maximized through the use of truncated BrAC measurements, as is commonly practiced in many jurisdictions. These findings mirror those observed using other evidentiary breath alcohol analyzers used in North America (7,8), Sweden (11), and New Zealand (9,10).

To examine the effect of a potential overestimation of the true vBAC, the truncated BrAC-vBAC differences were recalculated assuming a 5% and 10% overestimation of vBAC. In the first case (i.e., 5% overestimation), the difference between truncated BrAC and vBAC ranged from –27 to –1 mg/dL with mean and median values of –9 and –7 mg/dL, respectively. In the second case (i.e., 10% overestimation), the difference between truncated BrAC and vBAC ranged from –18 to +5 mg/dL

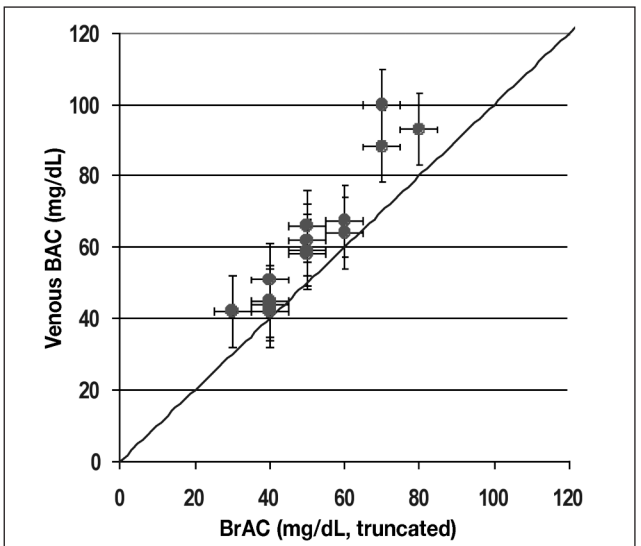

Figure 2. Plot of venous BAC (vBAC) measured using the enzymatic assay versus corresponding truncated BrAC measurements. Error bars represent the maximum SD observed in replicate measurements of vBAC and BrAC (10 mg/dL and 5 mg/dL). The solid line represents equivalence of vBAC and BrAC (i.e., vBAC = BrAC).

with mean and median values of –3 and –1 mg/dL, respectively. In our view, the likelihood of a systematic 10% overestimation is quite low because whole blood was used in assay calibration and was assayed directly from subject blood samples. Thus, we believe that the BrAC measurements made by with Intoxilyzer 8000C tend toward an underestimation of the actual BAC in light of these data. However, by considering the effect of various degrees of overestimation of the vBAC, the effects of the variability associated with measurements made by the enzymatic assay may be examined as well. Ideally, this work will be replicated using larger sample sizes in both laboratory and field settings and using headspace GC as the analytical method for blood alcohol analysis for improved analytical precision. Nonetheless, the data in Table IV correspond to observed blood/breath ratios averaging 2359 ± 288 with minimum, maximum, and median values of 1969, 3060, and 2300, respectively. These data are in good agreement with those of Jones and Andersson (11) and Gainsford et al. (9).

Conclusions

Overall, the Intoxilyzer 8000C was shown to be able to provide precision that is well within the standard limits currently adopted in Canadian jurisdictions. This precision appeared to be insensitive to breath sample volume over the 2–3 L range in the subjects examined here based on these limits. Further, the truncated breath alcohol measurements using the Intoxilyzer 8000C reliably underestimated the venous BAC in these samples by an average of 17.4% (12 mg/dL). Although the INVALID SAMPLE message was valuable in some cases (5 out of 23) for indicating the presence of residual mouth alcohol, it is clear that a mandatory delay before breath testing commences should be enforced to minimize the likelihood of falsely elevated BrAC measurements.

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