Determination of ethanol in micro-volumes of blood by headspace gas chromatography: Statistical comparison between capillary and venous sampling sites.

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Abstract:

Ethanol is the most commonly encountered drug in forensic toxicology with widespread use throughout society. For this reason, it is important that there are a variety of reliable and robust methods to detect and quantify the content of alcohol in the blood samples of suspected drink drivers. A common method of detection is GC-FID with a number of sample preparation techniques employed. Typically, venous blood is sampled and used in the analysis, however there is currently no legal specification in UK of the blood sample source. This study investigates the use of capillary blood as an alternative to venous blood alongside two different sample volumes: 100µL and 10µL. Venous and capillary blood were collected from volunteers who had consumed alcohol, all blood sampling was carried out 1 hour after cessation of drinking. The results show a statistically significant difference between venous and capillary samples with an average difference of 3.38±1.99 mg/100mL at 100µL and approximately 4.13±2.42mg/100mL at 10µL. Predominantly venous blood was detected at higher concentrations than the corresponding capillary samples. The deviations in alcohol samples from venous to capillary blood is consistent with previous studies, however, our research indicates that capillary blood is a viable matrix to test for alcohol albeit one that underestimates blood alcohol content in relation to venous sampling. There was no statistically significant difference between the 100µL and 10µL sample preparation methods on an individual basis, which infers that micro volumes of alcohol are suitable for a forensic blood alcohol analysis.

Keywords: Toxicology, Forensic Science, Blood alcohol, Capillary blood, Road Traffic Act

Introduction:

Ethanol (also known as alcohol, ethyl alcohol) is a widely used recreational drug worldwide. A survey carried out in Britain in 2017 indicated that 57% of respondents admitted to using alcohol recreationally, which equates to approximately 29.2 million people nationwide[1]. Ethanol acts as depressant on the central nervous system and produces effects of relaxation, sedation, loss of inhibitions and impairment of motor coordination[2]. Due to its effects and prominence in society, drink driving limits were introduced to improve roads safety and reduce road traffic collisions[3]. Alcohol limits in England and Wales were set at 80mg/100mLof blood, 35µg/100mL of breath and 107mg/100mLof urine[4]. On the 10th of April 2015 the statutory option for drink driving was removed (8 subsection 2 of the Road Traffic Act 1988). Initially this act stated that if a breath specimen contained no more than 50µg/100mL ethanol, then the breath sample can be replaced with a sample of either blood or urine and should an individual provide such a specimen then the original breath specimen will be discarded[3, 5-7]. This option was originally brought in to compensate for issues with the reliability of the alcohol reading in breath samples. However, a review of the drink and drug driving laws by Sir Peter North in 2010found that due to the increasing accuracy of evidential breath analysers the statutory option was unnecessary, and that an evidential breath reading alone is sufficient to ensure a conviction[3].

Since the publication of the North Report the technique most commonly used to detect alcohol in road traffic cases in the UK is the evidential breathalyser[3]. Typically, a preliminary roadside test is carried out which if failed requires a further evidential breath test to be conducted under arrest at a police station. This involves the provision of two confirmatory breath specimens, the lowest of which is utilised[5-7].Despite the breath alcohol limit being35µg/100mL a prosecution limit of 40µg/100mL is routinely used[3]. Moreover, the UK government is in the process of implementing mobile evidential breathalysers, which would mean gathering evidence at the roadside, without the need to go the police station to perform a final evidential breath test[8, 9]. However, frequently there are issues with either the operation of the evidential breathalysers or of the defendant's ability to provide a breath sample, for

example in 2017, in the UK alone, 3862 people involved in road collisions refused or failed to give breath samples[10]. In such cases the police can charge with failing to provide, or, more frequently, request a urine or blood sample. This is also the procedure that is followed if there is an issue with the operation of the evidential breathalyser. As a result, there is still a significant number of road traffic cases that require the analysis of blood and urine to secure drink drive convictions. In circumstances where blood samples are collected, this requires a forensic medical examiner or a trained healthcare professional. The process of collecting venous blood for toxicological analysis may be invasive, time consuming and difficult to achieve safely with an intoxicated, uncooperative suspect. Venous blood samples should be approximately 10mL in volume and divided into two separate samples, one of which is offered to the suspect as their B sample[11, 12]. The collection of venous blood is carried out as arterial blood alcohol concentrations (BAC) are higher during the absorption phase compared to venous blood, while during the elimination phase arterial blood alcohol is lower than venous blood alcohol, furthermore, the puncturing of arteries is not recommended[13].

A potential alternative sample matrix is capillary blood, this is less invasive method of sampling blood taken commonly from a puncture on the finger. Capillary blood is a mixture of venous and arterial blood[14]. However, is not presently utilised for analysis of ethanol in road traffic toxicology as the relationship between alcohol levels in capillary blood compared to venous blood is not well defined with only a limited number of studies conducted to date[15,16]. The most commonly utilised laboratory technique for the detection of alcohol is gas chromatography with flame ionisation detector (GC-FID)[17-19]. This technique is rapid, reliable and does not require any significant sample preparation or extraction[20].

With the increasing sensitivity of analysis due to advances in instrumentation smaller volumes of samples can be used. This includes micro sampling and alternative biological samples which are the subject of ongoing research in forensic toxicology[21, 22]. The benefits of these methods could include a less invasive and faster sample collection process along with a requirement for smaller sample volumes. Microsamples for analysis of blood alcohol has previously been demonstrated using

volumes as low as 20-50µL with 1H-NMR[23]and GC-FID[24, 25]. However, frequently casework laboratories tend to use larger volumes for GC-FID due to issues with intra sample uncertainty, with typical samples volumes of up to 0.1-1mL analysed.

The aim of this study was to examine the relationship between capillary and venous blood alcohol and to investigate whether capillary blood could act as an alternative to venous blood sampling. The use of capillary blood samples could lead to a reduction in sampling times and a simpler more efficient sample collection process. Due to the relatively small volume of capillary blood samples an effective analysis will require a micro sampling technique to analyse as little as 10µL of blood.

Materials and Methods:

Reagents and Materials

Aqueous ethanol standard solutions at concentrations of 10, 20, 50, 100, 200 and 400 mg/100mL from Cerilliant were used. Aqueous ethanol Certified Reference Material Quality control (QC)solutions at concentrations of 20, 80 and 200mg/100mL from LGC European Reference Materials acted as quality control samples. Anhydrous tertiary butanol, sodium metabisulfite from Fisher Scientific were used as internal standard and antioxidant respectively. The vials used for collecting and storing blood were 5mL LABCO vials with sodium fluoride/ potassium oxalate for venous blood and 300µL SARSTEDT MicrovetteCB 300 K2E tubes with EDTA dipotassium salt for capillary blood.

Internal standard was made by using 500mL distilled water, adding 25µL of tertiary butanol and 2.5g of sodium metabisulfite.

Study design

The study protocol was approved by The Faculty Research Ethics Committee (FREC) of Kingston University London, Ethics code: 1819063.1. All participants provided informed consent and signed consent forms to take part in the study. The volunteers were healthy individuals accustomed to social and moderate drinking: volunteers were aged between of 20-45 years, with height ranges from 165-185cm, and weight ranges 60-100kg. Prior to commencing the experiment all participants were not

monitored. No instructions were given what and when they could eat, drink (with the exception of alcohol) before start of the study.

During the study the participants were given the choice of two different alcoholic beverages, either a beer of 4.8% alcohol by volume (ABV) or a pre-mixed gin and tonic at 5% ABV, male volunteers chose to consume the beer whilst the female volunteers selected the gin and tonic mix. The male participants were provided with a volume of 568mL or 1136mL of beer, while female participants were provided with a volume of 250mL or 500mL of the gin and tonic mix. Participants completed drinking within a 40 minute period. The blood samples from participants were collected one hour after cessation of drinking. Throughout the study until completion of sample collections, participants were instructed not to drink, eat, urinate or smoke. Samples 1-3,5,7,22-31,34,38,39 were males who drank two 4.8% ABV pint measures (568mL) of beer, while samples 6,10-19,21,33,35,36,40 were male participants who drank one 4.8% ABV pint measure (568mL) of beer. Sample 8, 9,20,32,37 were female participants who drank two 250mL measures of gin and tonic, 5% ABV, while sample 4 was a female participant who drank one measure of 250mL gin and tonic 5% ABV.

Before taking the blood sample the sampling area was disinfected with wipes containing isopropanol. Approximately 5mL of whole blood was taken from an antecubital vein in a seated position using a disposable BD vacutainer safety-lok blood collection set with an attached vial holder and collected into 5mL LABCO vials with sodium fluoride and potassium oxalate preservative. These vials contain a minimum of 1% sodium fluoride and potassium oxalate. The vials and preservatives used in this study are the same make and manufacturer as those contained with the road traffic sample collection kit as used by polices forces in England. Approximately 3-5mL of blood was collected with a total vial capacity of 5mL.Capillary samples were obtained by using a disposable lancet to draw blood. This was taken from the index finger, this area was disinfected using isopropanol wipes prior to sampling and the lanced site was palpated to aid blood flow during the sample collection process. Two CB300K2E tubes amounting to approximately 600µL was taken per participant, this volume was required to ensure there was sufficient sample for duplicate analysis in 100 and 10µL batches. Once

sample collection was complete the samples were analysed by GC-FID within 24 hours of collection. Capillary blood was transferred from the original microvette containers into sealed 1.5ml glass vials, using glass Pasteur pipettes. The samples were then pipetted into headspace vials using Gilson microman M100 or M10 positive displacement pipettes and tips. Excess sample were used for repeat analysis in circumstances where QC's fell outside the acceptance range. Once sample analysis was complete samples were destroyed as per HTA guidelines.

For the first half of samples (Sample 1-18) venous blood was taken first followed by capillary blood, while for the remaining samples (Sample 19-40) the capillary blood was taken first followed by venous blood. This was done to determine if the delay associated with venous sampling before capillary blood would affect the difference between venous and capillary blood alcohol. Venous and capillary blood samples were taken from the same arm during the blood draw, with capillary blood being taken from index finger as well as ring finger if the required amount of blood was not collected from one finger.

There was a total of 13 volunteers used in this study with a number of volunteers providing more than one sample in separate sampling days, of the volunteers 8 were male with 5 being female. Of the male volunteers, 7 were Caucasian with the remaining one being of Asian descent, the Caucasian volunteers were primarily from the United Kingdom, the republic of Ireland and Eastern Europe, the Asian volunteer was of Bangladeshi origin. Of the female volunteers, one volunteer was of Middle Eastern origin, one was of Asian origin and three were of Caucasian origin, these volunteers were of Iranian descent, Irish, Sri Lankan, and Italian descent.

Sample preparation

Calibrants, samples, and QCs were made by pipetting 1mL of internal standard using an Eppendorf research pro 50µL-1mL electronic pipette to 20mL headspace vials and spiking it with 100µL of sample or calibrator or QC using a Gilson microman M100 positive displacement pipette. Aqueous quality control samples were run after calibration end and at end of each batch with concentrations of

20, 80 and 200mg/100mL. For micro-sampling 100µL of internal standard using the same automatic pipette as the 100µL batches was used and spiked with 10µLof either sample or calibrant or QC using a Gilson microman M10 positive displacement pipette. All samples were run in duplicate, using split flow with two columns and two detectors. Four quantitative values per sample were obtained.

Instrumentation

Shimadzu GC-2014 with RTX BAC 1(30m with 0.32mm ID) and RTX BAC 2 (30m with 0.32mm ID) dual column with HTA 200 H headspace auto sampler. Helium carrier gas, hydrogen FID fuel source, blank air to maintain FID flame ignition and nitrogen makeup gas. The GC-FID and headspace parameters are shown in Table 1.

Data analysis and Statistical analysis

The data analysis for the calibration curves, QCs and sample concentrations was carried out by Shimadzu GC solutions software. Microsoft excel was used to carry out statistical analysis using the formulas of average, stdev.p, t-test. A paired two tailed T-test was employed to check the significance of differences between the mean values, with values below or equal to 0.05 indicating a significant difference between means. Values were compared on an individual basis comparing the duplicate values of each sample (a total of N=4 measurements and 3 degrees of freedom). The entire sample population data was analysed using SPSS software. The population data was tested with Kolmogorov-Smirnov test for normality. For sample subsets that were not normally distributed a non-parametric test, Wilcoxon matched-pair signed rank test, was used to analyse the significance of difference, with value of $p<0.05$ indicating a significant difference. Coefficient of variance (CV) was used as a measure of variability as a high CV typically equates to a high variation of duplicate values in relation to the standard deviation and the mean. Standard deviation was calculated using the function of $\sigma = \sqrt{\frac{\sum(X-\mu)^2}{n}}$ whereσ is population standard deviation, $\sum$ is the sum of; µ the population mean and n the number of values within the data set. Coefficient of variance was calculated using the function: $CV = \frac{SD}{Mean} x\ 100\%$.

Results:

Investigation of the effects of reduced sample volume on the quantitation of blood alcohol in venous and capillary samples.

Analysis of blood alcohol concentration was carried out on samples of 100µL of venous blood and capillary blood. The analysis was then repeated on the same samples, with the sample volume reduced to 10µL. All calibration curves had a R^2 value of greater than 0.999 and all QC's were within 3% of certificate of analysis value. All CV's and SD were below 3% for all QC's with the exception of QC 20mg/100mL where SD alone was a more appropriate measurement. Our results indicate that a tenfold reduction in volume from 100µL to 10µL produces no statistically significant difference in the measured alcohol value in either venous or capillary blood samples on an individual sample by sample basis. However, a statistically significant difference was found for the differing sample volumes of capillary blood when comparing the entire sample population. Despite this, on an individual sample by sample comparison the BAC of 74% of samples, were not significantly different.

The statistically significant difference in the overall sample subset for capillary 100µLcompared to capillary 10µLvolumes, is due to the consistent trend in which the 100µL samples have a higher measured BAC. However, the average mean difference between the two sampling volumes was found to be just0.41mg /100mL in venous and 1.21mg/100mL in capillary blood samples. The values of CV and SD were similar for the different sampling volumes (Table 2and Table3), with the average CV for all 10µL volume samples being 2.87%, while for all 100µL volume samples it was 3.76%. This suggest that sample volumes as low as 10µL are still able to accurately quantify ethanol content in both venous and capillary blood and indicates that methods utilising reduced sample volume can perform at the same standard as the traditional higher sample volume methods.

Comparison of venous and capillary blood using standard sample volumes and reduced sample volumes

The differences in ethanol concentration for venous blood sampling and capillary blood sampling were investigated using sample volumes of 100µL and 10µL (Table 4 and Table5). Our results indicate that there was a statistically significant difference in alcohol concentration of capillary blood samples compared to venous blood samples, regardless of what sample volume was analysed. Aliquots of 100µLof venous and 100µLof capillary blood from the same donor sampled at the same time was analysed for alcohol. The average concentration difference was found to be 3.38mg/100mL, higher in venous blood. For the 10µLsample aliquots, the average blood alcohol concentration was 4.13mg/100mL higher in venous blood. The range of variation for the 100µLaliquots was0.58mg/100mL to 7.44mg/100mL. For the 10µLsample volumes the range of variation was 0.23mg/100mL to 10.94mg/100mL. All venous samples had higher ethanol concentrations than their corresponding capillary samples with the exception of samples 15 & 27 (Table 4). In these samples, capillary ethanol was greater by 1.01 and 0.93mg/100mL respectively, however, both of these increased concentrations are within normal analytical variation limits.

Discussion:

Our results indicate that the use of micro-sampling and reduced sample volume does not affect the accuracy of alcohol quantitation in blood samples when tested by GC-FID. It should also be noted that alcohol in capillary blood samples, was quantified on average, 3.76 mg/100mL lower than their corresponding venous samples. This raises the possibility of capillary blood samples and a corresponding micro sample analysis method being used in the course of road traffic toxicology casework, where rapid sampling and high accuracy quantitative analysis is required. Although it is clear that capillary samples may present an underestimation of the motorists blood alcohol concentration, this is offset by the more rapid collection procedure. The typical elimination rates of alcohol are between 15-25mg/100mL per hour, therefore any significant sampling delay can lead to an underestimation of the suspect's blood alcohol concentration at the time of incident[26]. In addition to this, one of the major obstacles to obtaining a blood sample is a suspect stating a fear (real or contrived) of needles and/or a failure of the healthcare professional to successfully extract the

required sample blood. While urine sampling is an alternative, the procedure is time consuming, and requires more manpower from law enforcement as the suspect has to urinate first and then provide an evidential urine sample within one hour. In addition to urine providing a less contemporaneous toxicological perspective than blood, the road traffic urine procedure is frequently subject to legal challenges[27]. Therefore, the preference for most law enforcement agencies in the UK is to collect blood where possible, suggesting that capillary blood extraction and micro sampling analysis could offer a valuable alternative to the current practices.

Previous research investigating micro sample testing of blood alcohol at lower sample volumes suggested this is a viable technique[23-25]. Wilkinson *et al* described a Headspace GC-FID method for ethanol analysis utilising 20-50µL blood samples with a reported average precision of 4.6% and a concentration range of 0.003-1.2 mg/mL[24]. A study by Vance *et al.* investigating GC-FID analysis of blood alcohol, utilised a 50µL sample volume with a 1mL internal standard volume and an acceptance criterion that the duplicate ethanol results must be within 5% or 5mg/100mL[25].Our results detail the CV and intra sample variation of 10µLvolumes of venous and capillary blood. The CV of 10µL venous blood samples was 2.54% and the CV of 10µL capillary blood samples was 3.21%. Neither of the methods employed by Vance *et al* and Wilkinson *et al.* compare the accuracy or viability of micro sampling with traditional sample testing, however, they do demonstrate the validity of the technique and support the results of this experimentation[24,25].An alternative method for blood alcohol concentration analysis employing 1H NMR was developed by Zailer and Diehl, where they utilised 20µL of blood for analysis of alcohol with their analysis method examining concentrations within the range of 0-3g/L[23]. While this method displayed an impressive sensitivity on a relatively small sample, the use of 1H NMR for volume toxicology analysis is not cost effective or commercially viable.

In addition to investigating the viability of reduced sample volumes in the analysis of blood alcohol concentration we also examined the differences between venous and capillary blood one hour after cessation of drinking. The data shows a statistically significant difference ($p \leq 0.05$) between the blood alcohol concentration of venous and capillary blood samples, almost all capillary blood samples

measured alcohol content was lower than that of the corresponding venous blood sample. This applies for individual aliquots as well as the full population data (Table 6). An average venous versus capillary difference of approximately 3.42 ±1.96mg/100mL at 100µl and approximately 4.29±2.29 mg/100mL at 10µl was observed. Previous work carried out by Jones *et al.* examined the differences in alcohol content of venous blood compared to capillary blood, and they focused on how different sampling times and source of blood (capillary or venous) influenced blood alcohol concentration [15]. This study suggested that whilst in the absorption phase, capillary blood alcohol was higher than the corresponding venous blood alcohol concentration. However, once the post absorptive phase is reached (after approximately 90 minutes), the venous blood alcohol was higher. The average capillary-venous difference of 5.8±3.4 mg/100mL which did not appear to change significantly for the remainder of the 390-minute experimentation period. The post absorptive capillary and venous blood alcohol variation described by Jones *et al*, is comparable to the results in this study. This suggests that there is a reliable correlation between the two blood sources. Jones *et al.* compared alcohol levels in venous and capillary blood one hour after the start of alcohol consumption, while this study looked at differences one hour after cessation of drinking, as one hour is an approximate time of the completion of the alcohol absorption phase [15,28,29]. Furthermore, this sampling procedure better reflects the process of collecting samples for road traffic toxicology analysis where sample collection only occurs after at least one hour has elapsed since the suspect last consumed alcohol.

A limitation of this study was the use of only one time point for sampling, utilising more time points, perhaps one before the hour at 30 minutes post consumption and a third time point at 90 minutes after consumption. This would allow for a more comprehensive analysis of the venous and capillary profile and further corroborate the work of Jones *et al.* regarding the venous and capillary difference during and after the absorption phase. A further limitation of this study was the lack of high BAC readings to compare the difference in venous versus capillary BAC at higher ranges in relation to lower levels. The addition of volunteers with BACs in excess of 80 mg/100mL would provide a better understanding of the relationship of BAC to the venous and capillary blood alcohol and this will be expanded upon in future research. Another limitation of this study and the subject of further research

is the lack of demographic variation of volunteers in this study. Moreover, a higher number of analyses with a larger number of participants would help to verify this proof of concept. By increasing the sample size of the participants this would further establish and confirm the statistical uncertainty and accuracy of utilising capillary blood for BAC analysis.

A potential difficulty of using capillary blood is extracting sufficient quantities for traditional analysis techniques, which typically use 0.1-1mL of sample in duplicate. Capillary blood, whilst easier to obtain in small volumes becomes more difficult when larger volumes are needed. The difficulty is however mitigated by utilising a 10µL sample volume which reduces the volume of sample required for an analysis whilst maintaining a comparable sensitivity to higher sample volume analyses.

The varied nature of the differences between capillary and venous values in this experimentation could be attributed to an unstandardized specification on fasting state and food intake prior to alcohol ingestion, as in this study volunteers were not required to fast before the experiment began. This could have played a role as volunteers would have a varied speed of gastric emptying, with some volunteers having eaten hours before the experimentation and some having not eaten for an extended period of time by comparison. Different meal compositions may also have played a part in this, with higher carbohydrate or fatty foods being a contributor to a slower gastric emptying rate. This could alter the time taken for the post absorptive phase of ethanol to be reached [28,29]. This was an intentional feature of the study in order to provide a cross sectional analysis of a simulated real life scenario, whereby suspects caloric and dietary intake will be varied on a case to case basis thus increasing inter subject variability. This has the consequence of raising pre-analytical variability, a further cause of the variation is that volunteers also consumed different volumes of different alcoholic beverages with some consuming just one pint and others consuming a second thus giving a higher variability of BAC values.

In conclusion, it has been found that a sample size of 10µL is a viable method of sampling for both venous and capillary blood samples. A plausible benefit of using micro samples for blood alcohol analysis is that there is the potential for a rapid, simpler and more efficient collection procedure from

detainees in custody. There is also no specification in UK law on where blood samples should be collected from on a drink driving suspect [30, 31]. Therefore capillary blood could be lawfully collected and analysed for blood alcohol concentration. Our results indicate that there is average 3.85mg/100mL increase in alcohol concentration for venous samples compared to capillary blood samples one hour after cessation of drinking. Therefore while faster collection times using capillary blood may be of benefit in detecting blood alcohol prior to elimination, they are likely to provide some underestimation of alcohol content when compared to venous blood. Regardless, this technique may still be useful in cases of poor venous access or inability of the patient to provide a venous sample.

Author contributions:

L.T, V.R, performed laboratory analysis and composition of the paper. L.S, J.M and T.B carried out laboratory analysis. C.H, H.M and J.B completed collection and designed sample collection procedure. B.T and S.C contributed to composition of paper. G.T and B.R responsible for study design and principal investigators.

Declaration of conflicting interests:

The authors declare that there is no conflict of interests

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Table 1: Gas chromatography and headspace sampler parameters for the analysis of ethanol in blood. The method was designed specifically for quantitation of ethanol in blood samples. This method was validated prior to the study initialisation.

Parameter	**Value**	
Inlet Temperature	110 ºC	
Injection mode	Split	
Pressure	85 Kpa	
Column Flow	2.78 ml/min	
Linear velocity	42.30 cm/sec	
Purge flow	3.00 ml/min	
Split ratio	5.00	
Oven temperature	40 ºC isothermal	
Oven temperature (Headspace sampler)	60 ºC	
Syringe temperature	70 ºC	
Fill volume	1.75 mL	
Oscillation time	0.50 minutes on	0.10 minutes off
Sample speed	5.0 mL/min	
Injection speed	80 mL/min	
Sample speed	5.0 mL/min	

Table 2: Comparison of blood-ethanol concentrations determined using 10 µL or 100 µL aliquots of venous whole blood. Our results indicate that a tenfold dilution in sample volume does not result in significant difference in detected alcohol concentration of this sample matrix.

Sample	Mean BAC for 100µl volume (mg/100mL)	SD	CV%	Mean BAC for 10µl volume (mg/100mL)	SD	CV%	Difference (mg/100mL)	Paired t-test P value
1 (M)	65	1.45	2.23	64	2.29	3.56	0.73	0.649
2 (M)	74	1.33	1.79	75	1.07	1.43	0.29	0.820
3 (M)	56	0.99	1.77	56	2.20	3.90	0.69	0.645
4 (F)	7*	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5 (M)	57	0.37	0.65	58	0.72	1.24	0.96	0.194
6 (M)	20	0.45	2.23	20	0.23	1.12	0.35	0.128
7 (M)	67	0.78	1.17	71	0.32	0.45	3.52	0.005
8 (F)	26	0.60	2.33	27	1.26	4.65	1.29	0.245
9 (F)	26	0.54	2.07	27	0.45	1.67	0.93	0.009
10 (M)	23	0.99	4.29	21	0.52	2.45	1.89	0.037
11 (M)	19	1.28	6.63	19	0.92	4.86	0.42	0.254
12 (M)	17	1.44	8.61	16	0.72	4.64	1.18	0.101
13 (M)	28	0.51	1.83	28	0.79	2.87	0.09	0.742
14(M)	31	1.12	3.60	30	1.22	4.05	0.88	0.399
15(M)	19	2.17	11.48	19	0.55	2.91	0.01	0.606
16(M)	27	1.29	4.81	27	0.73	2.68	0.44	0.629
17(M)	25	0.81	3.24	26	1.07	4.17	0.52	0.335
18(M)	29	1.16	4.01	29	1.17	4.03	0.01	0.984
19(M)	25	0.97	3.81	23	0.97	4.25	2.49	0.038
20(F)	31	1.42	4.62	30	0.07	0.25	1.10	0.485
21(M)	25	1.55	6.19	27	1.04	3.87	1.75	0.299
22(M)	64	0.37	0.58	62	0.74	1.19	2.31	0.392
23(M)	58	1.11	1.90	52	1.11	2.13	6.08	0.007
24(M)	67	1.02	1.52	62	0.70	1.13	5.21	0.087
25(M)	59	1.22	2.07	58	1.01	1.74	0.61	0.435
26(M)	76	0.70	0.92	74	1.41	1.89	1.47	0.195
27(M)	72	0.84	1.17	73	1.88	2.60	0.41	0.531
28(M)	42	1.88	4.44	43	0.68	1.57	0.91	0.563
29(M)	64	2.33	3.66	62	1.13	1.81	1.51	0.496
30(M)	64	1.41	2.20	67	2.81	4.18	2.75	0.224
31(M)	63	2.26	3.57	65	1.16	1.80	1.35	0.365
32(F)	32	1.92	6.07	33	0.79	2.36	1.76	0.133
33(M)	21	1.01	4.74	20	0.59	2.88	1.09	0.322
34(M)	73	0.46	0.63	72	1.01	1.41	0.74	0.220
35(M)	31	0.27	0.84	30	0.09	0.30	1.56	0.011
36(M)	21	0.55	2.60	18	1.15	6.32	2.85	0.077
37(F)	35	0.22	0.63	35	0.34	0.97	0.56	0.350
38(M)	65	0.88	1.34	66	0.68	1.03	1.28	0.258
39(M)	63	1.22	1.93	62	1.14	1.83	0.73	0.659
40(M)	36	1.06	2.97	33	0.96	2.92	2.77	0.009

**Value was below the limit of quantitation of the method* *M=male F=female*

Table 3: Comparison of blood-ethanol concentration determined using 10 µL or 100 µL aliquots of capillary (fingertip) blood. Capillary whole blood samples were analysed at two different volumes. Our results indicate that dilution of the sample volume from 100 µL to 10 µL does not result in a significant difference in reported alcohol value of this sample matrix.

Sample	Mean BAC for 100µl volume (mg/100mL)	SD	CV%	Mean BAC for 10µl volume (mg/100mL)	SD	CV%	Difference (mg/100mL)	Paired t-test P value
1 (M)	61	1.24	2.03	60	1.62	2.69	0.76	0.579
2 (M)	69	1.29	1.88	67	1.67	2.51	2.15	0.028
3 (M)	51	1.90	3.76	48	0.54	1.12	2.45	0.119
4 (F)	6*	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5 (M)	52	0.83	1.58	55	1.23	2.25	2.44	0.072
6 (M)	14	0.58	4.11	15	0.34	2.23	1.21	0.039
7 (M)	60	1.06	1.79	60	0.65	1.08	0.68	0.404
8 (F)	20	0.59	2.93	21	0.48	2.31	0.68	0.148
9 (F)	21	0.35	1.71	21	0.27	1.30	0.39	0.070
10 (M)	19	0.73	3.87	16	0.65	4.00	2.64	0.000
11 (M)	14	0.91	6.43	12	0.72	6.30	2.63	0.098
12 (M)	11	1.05	9.19	9*	0.84	9.01	2.02	0.005
13 (M)	25	1.26	4.95	26	1.88	7.33	0.14	0.942
14(M)	29	1.46	5.10	27	1.24	4.59	1.68	0.140
15(M)	20	0.89	4.48	19	0.50	2.68	1.25	0.094
16(M)	25	1.35	5.36	24	0.96	4.03	1.34	0.031
17(M)	23	1.06	4.57	23	0.88	3.90	0.60	0.150
18(M)	26	0.78	2.99	24	1.58	6.57	2.12	0.378
19(M)	21	0.13	063	21	1.34	6.46	0.03	0.197
20(F)	29	0.35	1.19	27	1.09	4.10	2.68	0.086
21(M)	22	0.65	2.98	21	1.24	5.84	0.56	0.726
22(M)	61	0.07	0.11	57	0.99	1.75	4.66	0.024
23(M)	54	0.00	0.02	51	1.83	3.60	3.32	0.153
24(M)	62	0.56	0.90	60	0.54	0.90	2.31	0.034
25(M)	54	0.71	1.33	52	0.85	1.63	1.37	0.134
26(M)	71	0.89	1.25	69	2.11	3.08	2.14	0.157
27(M)	73	0.67	0.91	71	0.91	1.29	2.05	0.077
28(M)	39	2.34	5.95	41	1.58	3.83	1.80	0.704
29(M)	58	1.71	2.98	58	0.48	0.84	0.34	0.240
30(M)	61	0.70	1.15	64	1.02	1.59	2.97	0.010
31(M)	61	3.56	5.83	59	1.49	2.50	1.82	0.288
32(F)	29	1.11	3.83	30	2.03	6.84	0.66	0.584
33(M)	21	0.49	2.38	19	0.52	2.74	1.89	0.127
34(M)	68	0.94	1.38	67	0.33	0.48	0.91	0.255
35(M)	30	0.77	2.53	29	0.17	0.60	1.93	0.122
36(M)	20	0.22	1.09	18	0.75	4.26	2.20	0.012
37(F)	33	0.36	1.11	28	1.07	3.78	4.29	0.028
38(M)	65	0.99	1.53	60	0.84	1.39	4.28	0.066
39(M)	60	1.06	1.75	57	1.58	2.75	2.94	0.283
40(M)	32	0.83	2.57	30	0.26	0.89	2.58	0.048

**Value was below the limit of quantitation of the method* *M=male F=female*

Table 4: Comparison of ethanol concentrations in samples of venous and capillary blood using 100 µL aliquots. Our results indicate that capillary and venous blood provide differing BAC values and cannot be treated as equivalent matrices. In approximately 92.5% of samples the corresponding capillary BAC was on average 3.38mg/100mL lower than the venous equivalent.

Sample	Mean BAC for 100µL venous (mg/100mL)	SD	CV%	Mean BAC for 100µL capillary (mg/100mL)	SD	CV%	Difference (mg/100mL)	Paired t-test P value
1 (M)	65	1.45	2.23	61	1.24	2.03	4.01	0.028
2 (M)	74	1.33	1.79	69	1.29	1.88	5.61	0.014
3 (M)	56	0.99	1.77	51	1.90	3.76	5.09	0.028
4 (F)	7*	n/a	n/a	6*	n/a	n/a	n/a	n/a
5 (M)	57	0.37	0.65	52	0.83	1.58	4.55	0.007
6 (M)	20	0.45	2.23	14	0.58	4.11	5.95	0.000
7 (M)	67	0.78	1.17	60	1.06	1.79	7.44	0.001
8 (F)	26	0.60	2.33	20	0.59	2.93	5.70	0.000
9 (F)	26	0.54	2.07	21	0.35	1.71	5.48	0.000
10 (M)	23	0.99	4.29	19	0.73	3.87	4.28	0.002
11 (M)	19	1.28	6.63	14	0.91	6.43	5.14	0.003
12 (M)	17	1.44	8.61	11	1.05	9.19	5.29	0.000
13 (M)	28	0.51	1.83	25	1.26	4.95	2.16	0.094
14(M)	31	1.12	3.60	29	1.46	5.10	2.46	0.034
15(M)	19	2.17	11.48	20	0.09	4.48	-1.01	0.552
16(M)	27	1.29	4.81	25	1.35	5.36	1.65	0.000
17(M)	25	0.81	3.24	23	1.06	4.57	1.85	0.036
18(M)	29	1.16	4.01	26	0.78	2.99	2.83	0.075
19(M)	25	0.97	3.81	21	0.13	0.63	4.64	0.114
20(F)	31	1.42	4.62	29	0.35	1.19	1.44	0.251
21(M)	25	1.55	6.19	22	0.65	2.98	3.34	0.037
22(M)	64	0.37	0.58	61	0.07	0.11	2.83	0.012
23(M)	58	1.11	1.90	54	0.00	0.02	4.30	0.012
24(M)	67	1.02	1.52	62	0.56	0.90	5.07	0.096
25(M)	59	1.22	2.07	54	0.71	1.33	5.15	0.019
26(M)	76	0.70	0.92	71	0.89	1.25	5.06	0.008
27(M)	72	0.84	1.17	73	0.67	0.91	-0.93	0.332
28(M)	42	1.88	4.44	39	2.34	5.95	3.04	0.082
29(M)	64	2.33	3.66	58	1.71	2.98	6.10	0.006
30(M)	64	1.41	2.20	61	0.70	1.15	3.17	0.014
31(M)	63	2.26	3.58	61	3.56	5.83	2.01	0.524
32(F)	32	1.92	6.07	29	1.11	3.83	2.53	0.010
33(M)	21	1.01	4.74	21	0.49	2.38	0.69	0.361
34(M)	73	0.46	0.63	68	0.94	1.38	4.74	0.002
35(M)	31	0.27	0.84	30	0.77	2.53	0.96	0.204
36(M)	21	0.55	2.60	20	0.22	1.09	1.20	0.013
37(F)	35	0.22	0.63	33	0.36	1.11	2.73	0.102
38(M)	65	0.88	1.34	65	0.99	1.53	0.58	0.766
39(M)	63	1.22	1.93	60	1.06	1.75	2.80	0.128
40(M)	36	1.06	2.97	32	0.83	2.58	3.42	0.015

**Value was below the limit of quantitation of the method* *M=male F=female*

Table 5: Comparison of ethanol concentrations in samples of venous and capillary blood using 10 µL aliquots. Our results indicate that micro analysis of capillary and venous blood provide differing BAC values, cannot be treated as equivalent matrices. In approximately 97.5% of samples the corresponding capillary BAC was on average 4.13mg/100mL lower than the venous equivalent.

Sample	Mean BAC for 10µL venous (mg/100mL)	SD	CV%	Mean BAC for 10µL capillary (mg/100mL)	SD	CV%	Difference (mg/100mL)	Paired t-test P value
1 (M)	64	2.29	3.56	60	1.62	2.69	4.05	0.168
2 (M)	75	1.07	1.43	67	1.67	2.51	8.05	0.015
3 (M)	56	2.20	3.9	48	0.54	1.12	8.23	0.007
4 (F)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5 (M)	58	0.72	1.24	55	1.23	2.25	3.07	0.044
6 (M)	20	0.23	1.12	15	0.34	2.23	5.08	0.000
7 (M)	55	0.68	1.24	44	0.58	1.33	10.94	0.000
8 (F)	27	1.26	4.65	21	0.48	2.31	6.31	0.002
9 (F)	27	0.45	1.67	21	0.27	1.30	6.02	0.000
10 (M)	21	0.52	2.45	16	0.65	4.00	5.03	0.000
11 (M)	19	0.92	4.86	12	0.72	6.30	7.35	0.008
12 (M)	16	0.72	4.64	9*	0.84	9.01	6.14	0.000
13 (M)	28	0.79	2.87	26	1.88	7.33	1.93	0.061
14(M)	30	1.22	4.05	27	1.24	4.59	3.27	0.000
15(M)	19	0.55	2.91	19	0.50	2.69	0.23	0.647
16(M)	27	0.73	2.68	24	0.96	4.03	3.43	0.008
17(M)	26	1.07	4.17	23	0.88	3.90	2.97	0.037
18(M)	29	1.17	4.03	24	1.58	6.57	4.94	0.047
19(M)	23	0.97	4.25	21	1.34	6.46	2.11	0.094
20(F)	30	0.07	0.25	27	1.09	4.10	3.02	0.052
21(M)	27	1.04	3.87	21	1.24	5.84	5.65	0.013
22(M)	62	0.74	1.19	57	0.99	1.75	5.18	0.014
23(M)	52	1.11	2.13	51	1.83	3.60	1.55	0.382
24(M)	62	0.70	1.13	60	0.54	0.90	2.17	0.039
25(M)	58	1.01	1.74	52	0.85	1.63	5.91	0.008
26(M)	74	1.41	1.89	69	2.11	3.08	5.74	0.006
27(M)	73	1.88	2.60	71	0.91	1.29	1.53	0.407
28(M)	43	0.68	1.57	41	1.58	3.83	2.16	0.106
29(M)	62	1.13	1.81	58	0.48	0.84	4.24	0.043
30(M)	67	2.81	4.18	64	1.02	1.59	2.96	0.148
31(M)	65	1.16	1.80	59	1.49	2.50	5.18	0.024
32(F)	33	0.79	2.36	30	2.03	6.84	3.63	0.038
33(M)	20	0.59	2.88	19	0.52	2.74	1.48	0.088
34(M)	72	1.02	1.41	67	0.33	0.48	4.91	0.032
35(M)	30	0.09	0.30	29	0.17	0.60	1.33	0.129
36(M)	18	1.15	6.33	18	0.75	4.26	0.55	0.513
37(F)	35	0.34	0.97	28	1.06	3.78	6.46	0.089
38(M)	66	0.68	1.03	60	0.84	1.39	6.14	0.095
39(M)	62	1.14	1.83	57	1.58	2.75	5.00	0.243
40(M)	33	0.96	2.92	30	0.26	0.89	3.23	0.032

**Value was below the limit of quantitation of the method* *M=male F=female*

Table 6: Wilcoxon matched-pair signed rank test for all subject data at each sample subset.

Nonparametric statistical analysis was used to analyse the statistical significance of BAC from differing sample sites and different blood volumes analysed. All sample subsets, with exception of venous 100µL blood compared to venous 10µL blood, were found to be significantly different.

Sample subset	Number of samples	Mean difference ± SD (mg/100mL)	95% CI for mean difference	Wilcoxon signed rank test P value
Comparison of BAC of 100µL and 10µL aliquots of venous whole blood	39	0.41±1.94	-0.22 to 1.04	0.209
Comparison of BAC of 100µL and 10µL aliquots of capillary whole blood	38	1.21±1.88	0.59 to 1.83	P=0.001
Comparison of BAC of 100µL and 100µL aliquots of venous and capillary whole blood	39	3.38±1.99	2.74 to 4.03	P<0.001
Comparison of BAC of 10µL and 10µL aliquots of venous and capillary whole blood	38	4.13±2.42	3.34 to 4.93	P<0.001