I. Table of Contents

II. INTRODUCTION

II.1. Purpose

This is Scottsdale Police Department Crime Laboratory's procedure for the analysis of ethanol in biological fluids using headspace gas chromatography with dual capillary columns and flame ionization detectors. One column is used for quantification, while the other is used as the confirming column.

II.2. Safety

a. Disposable plastic apron and/or other barrier cover(s), single or double disposable gloves; face shield or disposable mask, along with eye protection will be worn when working with blood or other biological samples.

b. All transferring of any potentially hazardous raw biological samples from one vial to another will be performed under a safety hood.

c. All disposable protective clothing and used headspace vials containing blood samples will be disposed of by placing them in the biological waste container which in turn will be removed from the lab on a regular basis for proper disposal.

d. For additional information, refer to FSD8110 Division Operations orders safety and chemical hygiene sections.

II.3. Security

a. Samples will be handled consistent with good forensic practice. Samples may only be left unattended in a secure area, i.e. the toxicology laboratory or evidence vault. If a visitor or service technician is present, samples must be attended or locked in a secure area.
b. Laboratory security is as outlined in FSD8110 and QSM 5.3.

III. Methods and Instrumentation

III.1. Instrumentation, Equipment, and Supplies

A. List of Instruments, equipment, and supplies

1) Perkin-Elmer Instruments:
   a. Model Clarus 500 Gas Chromatograph serial number 650N9042003 or 650N9042002.
   b. TurboMatrix 110 Headspace Sampler
   c. Total Chrom software version 6.3.2 or higher, to include TurboMatrix driver.
2) HP Laser Jet 4000 printer or comparable.
3) Dilutor/Dispenser: Hamilton Mircolab 530B or comparable.
4) Capillary Columns:
   a. Quantitative Column: PE Elite BAC 1, 30m x 0.32mm, method: "method2002A" or "method2003A" or equivalent.
   b. Confirmation Column: PE Elite BAC 2,30m x 0.32mm, method: "method2002B" or "method2003B" or equivalent.
5) Headspace Vials and Septa: 20 ml capacity with appropriate caps.
6) Mechanical or hand crimper for 20mm vials.
7) Ethanol: 200 proof anhydrous ethanol and externally prepared ethanol standards.

B. BA Instrument Operating Parameters

1) Clarus 500 GC:
   a. FID A and B:
      1. Set H_2 to 45.0 ml/min flow.
      2. Set Air to 450.0 ml/min flow.
   b. GC conditions:
      1. Detectors A and B: 250° C
      2. Injector: 150° C
      3. GC Oven: 38° C
      4. Split Ratio:10.0 ml/min
      5. Run time: 4.00 min.

2) TurboMatrix 110 Autosampler:
   a. The method has the following conditions:

1. Needle: 70°C
2. Transfer line: 80°C
3. Vial Oven: 60°C
4. Pressurization time: 1.0 min
5. Injection time: 0.03 min
6. Withdrawal time: 0.2 min
7. Thermostat time: 22.0 min
8. Cycle time: 4.0 min
9. Column head pressure: 16 psi
10. Inject Mode: Time
11. HS Mode: Constant

C. Microlab 530B Diluter Pipetter parameters

1) Programming Microlab 530B Diluter: The settings for the "BA" method are as follows:

Left syringe size (µl):	2500	
Right syringe size (µl):		250
Dilute method		
Ratio 1:		10.0
Dilution 1:		11.0
Left diluent volume (µl):		2500.0
Right air gap volume (µl):		5.0
Right sample volume (µl):		250.0
Final volume:		2750
Syringe fill speed, left:		3
Syringe aspirate speed, right:		2
Syringe dispense speed, left:		4
Syringe dispense speed, right:		2
Syringe fill mode:		Auto
Air gap mode:		Auto
Air gap delay:		0.1
Wash volume (µl):		1250.0
Left fill speed:	3	
Left dispense speed:	2	

D. Maintenance

1) Perkin-Elmer Clarus 500 and TurboMatrix 110. The GC and Headspace sampler will be maintained on a semi-annual preventative basis by a representative of the manufacturer. Any repairs or maintenance required outside of the regular

schedule will be performed as needed by a representative of the manufacturer. All repairs and maintenance records will be kept in the BA Maintenance Logs binder.

2) Microlab diluter-dispenser. This system will be assessed on a quarterly basis or more frequently if needed. This assessment will consist of accessing the MAINT program on the control panel and weighing 10 aliquots of room temperature purified water and determining the average and standard deviation of those measurements. Results will be charted and maintained in the BA Maintenance Logs binder. Additional evaluations using other dispensing volumes may be performed as needed. The current acceptable standard deviation based on 4 years of data acquisition on this type of system, is 0.033. Any check outside that will necessitate the pipettor being taken from service and repaired. The pipettors will be calibrated annually on a schedule with the other pipettes by an outside vendor.

3) Refrigerators. The Blood Alcohol section relies on two refrigerators; one for the storage of standards and one for the storage of subject samples in the laboratory. Both of these refrigerators contain a NIST traceable thermometer. The temperature logsheets are placed near the respective refrigerators and will be evaluated on a weekly basis. These logs will be collected when complete and maintained in the appropriate maintenance log binder. The temperature for the refrigerators will be maintained above freezing and below 8°C. If the temperature is outside of the acceptable range but is still cold, the analyst will adjust the temperature manually. If the appropriate range is still not attainable, the analyst will take the refrigerator out of service and move the samples or blood alcohol standards to another refrigerator within the lab which has an acceptable temperature as read on the thermometer. If the refrigerator is not cooling, it is to be immediately taken out of service and the blood or blood alcohol standards moved to a suitable refrigerator. Any time the refrigerator needs to be taken out of service, it will be recorded on the refrigerator log sheet.

E. Standard Solutions preparation parameters:

1) Ethanol Calibration Standards: 0.02, 0.10, 0.20, and 0.40% w/v
   a. Prepare 500ml of 10.0% w/v stock solution using absolute ethanol and DIW at room temperature.
   b. Using the 10% solution, prepare 500ml of each of the working solutions as follows:

Stock	Calibration Standards
1 ml	0.02% w/v
5 ml	0.10% w/v
10 ml	0.20% w/v

20 ml 0.40% w/v

2) Mixed Standard: (prepare in 1L)

Compound	% w/v	ml/L (pure solvent)
Acetaldehyde	0.02	0.25
Acetone	0.02	0.25
Ethanol	0.079	1.0
Methanol	0.08	1.0
Isopropanol	0.08	1.0

3) Internal Standard:
   1) Stock Solution: 15% w/v n-propanol (150 g/L).
   2) Working Solution: 0.015% w/v (1ml stock to 1 liter).
4) Add 0.05g/L $HgCl_2$ to the above stock solutions.
5) All working and stock solutions will labeled with the contents, lot number, and date prepared.

III.2. Methods. This procedure outlines the parameters required for successful and uniform completion of whole blood and control analysis for ethanol.

A. Sample Preparation (blood):

1) Remove one blood tube for analysis.
2) Label two headspace vials with DR# or suspect's name and vial number.
3) Before preparing each case sample check that the DR# and/or name on both vials correspond with DR# or name on blood tube.
4) Dispense 2500 µl n-propanol (0.015% w/v ISTD) and 250 µl of sample from blood tube into headspace vial, cap and crimp. Repeat for second vial.
5) Place prepared vials in rack in front of blood tube.

B. Sample preparation (other liquids)

1) Remove cap and determine if scent of liquid indicates alcohol. If not, the sample may be handled neat in the same way as a blood sample. If yes, then the sample should be diluted by a factor of 50 or 100 in purified water.
2) Label two headspace vials with DR# or suspect's name and vial number.
3) Before preparing each case sample check that the DR# and/or name on both vials correspond with DR# or name on sample tube.
4) Dispense 2500 µl n-propanol (0.015% w/v ISTD) and 250 µl of sample from container into headspace vial, cap and crimp. Repeat for second vial.
5) Place prepared vials in rack.

C. Calibration Standards Preparation:

1) Use the aqueous 0.02, 0.10, 0.20 and 0.40 % w/v ethanol standards made from a solution produced from 200 proof ethanol prepared as in 2.E.1 and verified against known traceable standards or equivalent commercially standards as verified in VI.2.
2) Label headspace vials with calibration standard level.
3) Dispense 2500 µl n-propanol (ISTD) and 250 µl of calibration standard into headspace vial, cap and crimp.

D. Control Standards Preparation: Blank, Control Reference Material (CRM), Mixed and Check Standards preparation:

1) The "Blank" is a vial containing Internal Standard.
2) A "CRM" is a solution containing a known amount of ethanol obtained from an independent outside source. This may be a water or whole blood matrix.
3) A mixed standard is a solution of ethanol and other volatiles in a water matrix to demonstrate analytical separation of possible components. Exact concentrations of components is not required.
4) Prepare single vials for the mixed standard and blank in the same manner as samples and calibrators.
5) The Check Standards are a series of at least three standards at varying concentrations to demonstrate the linearity of the calibration curve throughout. The check standards will be distributed throughout the sequence such that one high and one low check standard (≥ 0.2, ≤ 0.08) are evaluated before any unknown samples and also after all unknown samples. In the approximate middle of the unknown samples two check standards in the center range will be evaluated along with the whole blood standards, if present (if whole blood standards are not being used, two other CRMs of any concentration will be used at this point in the sequence). The center range is greater than or equal to 0.08 and less than or equal to 0.30.

E. Analysis sequence parameters

1) Place the vials in the TurboMatrix 110 in the following positions:
   - 1st: 0.02% Calibration Standard
   - 2nd:0.10% Calibration Standard
   - 3rd: 0.20% Calibration Standard
   - 4th: 0.40% Calibration Standard
   - 5th: Blank
   - 6th: Mixed Standard
   - 7th ,8th , last 2: Check standards as described above.
   - 9th, 10th, et al: Duplicate Case Samples.
2) Double check vial sequence in the TurboMatrix 110 tray before and after analysis.

IV. Case Documentation

All case files will contain at a minimum the analysts' laboratory notes and the subject chromatograms.

V. Reporting sample results

1. Whole Blood: Prepare as described in the above and report as "...analysis of the blood...".
2. Clotted Blood: Place contents of collection tube into a tissue grinder and grind sample, then prepare as above. Report as "...analysis of the blood...".
3. Serum, Plasma: Separate supernatant from cellular material, if present. Prepare as above and report that sample is improper and no determination of blood alcohol concentration will be made. Report presence or absence of alcohol. If a quantitative report must be issued, the analyst may either report the serum or plasma analyzed concentration and make it abundantly clear by the wording, use of all caps, and bold lettering, that the numerical value does not refer to whole blood. The analyst may also perform an appropriate conversion calculation and add it to the case notes.
4. Trace samples: Obtained values less than 0.020 BAC but greater than 0.010 BAC will be reported out as "Trace ethanol detected" or other similar phrasing. Samples where obtained value is less than 0.010 will be reported as "ethanol not detected".
5. Report the lower of the two case results truncated to three decimal places for the valid duplicate.
6. For liquid alcohol samples, the amount reported is the obtained value multiplied by the appropriate dilution factor. This number is reported as a % w/v as "...analysis of the liquid ..."

VI. Quality

1. Analysis Results Validation and Technical Review parameters:

A. Plot a calibration line. The calibration curve must have an R^2 value of ≥ 0.995 or data obtained using that calibration curve will not be reported. The calibration curve will consist of the four points listed in III.2.E.1.

B. The calibration curve will be valid for up to forty eight (48) hours.

C. The retention time of ethanol must be within 0.04 minutes of that of the calibration standards. If this criterion is not met the peak may not be reported as ethanol.

D. All CRM and Check Standard results must be within the greater of ± 5% or 0.005 g/dl of target value. If one or more valid CRMs returns a value of greater than 5% outside the target value, the reason will be assessed.

1) If the cause is a loose cap or some other obvious cause and the control is in the beginning or middle of the sequence, and remaining controls are within tolerance, there is no further action needed, as the results of the remaining controls can serve to

validate the ability of the instrument to accurately analyze samples for ethanol concentration.

2) If one of the controls at the end of the sequence does not meet the criterion due to a loose cap, it may be recapped and reanalyzed under the same calibration curve within the 48 hours. If the control then meets the requirements, no further action is needed and the sequence will be accepted as valid.
3) No more than 2 excused controls may be present in any single batch (which are not closing controls that can be reanalyzed) for the sequence to be considered valid.
4) If no valid or obvious reason exists for a Check Standard being out of control, the batch will be rejected and no sample values will be reported. The problem will be evaluated and corrected before further sample acquisition.

E. Results of duplicate case samples must be within 5% or 0.005 g/dL of each other (whichever is greater). If sample duplicates are not within tolerance, then the case sample will be analyzed again by either:

1) Using the original calibration curve and reanalyzing the case sample at the end of the original sequence run followed by at least 2 calibration check samples, or
2) Reanalyzing the sample using a new calibration curve and controls.
3) The results of all analyses will be recorded in the analysts notes.

F. A loose cap may be identified by the sample having an internal standard area count more than 25% lower than the average internal standard area count measured in the controls analyzed in the batch for that day. If this is the case for a control sample, it will be evaluated and addressed as in item VI.1.D, above. If it is a subject sample, it will be addressed as in item VI.1.E, above.

G. The chromatogram for the blank sample must not show the presence of any substance that could interfere with the quantification of ethanol. If any such substance is detected, results from the run will not be reported.

H. The chromatogram for the mixed standard must show separation of the volatile compounds contained therein. If peaks from all the volatiles known to be in the mix are not present in the chromatogram, results from the run cannot be reported until the cause is identified and addressed.

I. A second analyst must review and approve results of all analyses before results can be reported (100% technical review). This reviewer must have been previously qualified to conduct blood alcohol analyses within a forensic laboratory. Technical review will consist of ensuring that the entire sequence including control and subject samples meet the criteria presented in this section (VI.1). The meeting of these criteria will be signified by the initials of the technical reviewer on the face sheet for the sequence. The technical reviewer will also review all analysts' notes and results for accuracy and signify acceptance by approving electronically in the LIMS system. Any discrepancies between the technical reviewer and the examiner which cannot be reconciled between themselves may be brought to the attention of the Technical Leader or the Quality Manager for evaluation of compliance with current standards and practices.

2. Validation of working solutions:

A. Newly prepared ethanol calibration solutions will be verified by establishing that an acceptable calibration curve can be achieved using the new standard. The calibration must have an $R^2 \geq 0.995$.

B. Calibrators will additionally be verified by analyzing a series of known, externally prepared, NIST traceable standards covering the span of the calibration curve against the newly established calibration curve. All known standard calculated values must be within ± 3% of their true values or 0.003 g/dL, whichever is greater, for the calibrators to be acceptable for use.

C. Calibrators may also be sent out to another crime laboratory for verification of accuracy.

D. Newly prepared internal standard (ISTD) solutions will be evaluated by preparing and analyzing a blank sample using 2500 µl of the ISTD and demonstrating absence of any interfering compounds and an area count within ± 20% of the current ISTD lot. The individual preparing the ISTD is responsible for testing that lot of ISTD in duplicate and ensuring that it meets the criteria. The chromatograms and examiners initials and date prepared will be maintained in the Blood Alcohol "ISTD Verification" binder. If the internal standard does not meet this requirement, it will be discarded and reprepared. If there has been a significant change to the method or instrument such that this criteria cannot be met, it will be explained in the maintenance log and on the ISTD log.

E. The mixed standard is to establish qualitatively the ability of the method to separate a variety of volatile components which may be reasonably

expected to appear occasionally in a blood sample from a living human. Quantitative accuracy of the preparation is not required; however the prepared mix may be verified for retention time against an externally prepared mixed standard to ensure presence of all components.

F. Validation information for the calibrators and will be maintained in the "Calibrator Verification" book.

3. Validation of externally acquired alcohol standards

A. All externally acquired control standards in water or whole blood matrix will be validated before use by analyzing against the established calibration curve.

B. Analyzed concentrations must be within ± 3% or 0.003 g/dL, whichever is greater, of the target value (supplied by the manufacturer) for the standards to be put in to use.

C. Verifications of this validation will be maintained in the "QC Verification" book.

4. Uncertainty of Measurement

Estimation of uncertainty for blood alcohol analysis was evaluated in accordance with ASCLD/LAB International requirements in conjunction with ARS statutes and was determined to be necessary. Full explanation and information may be found separately in the binder labeled as 'SPD Blood Alcohol Uncertainty of Measurement". The general outline is as follows:

a) A previous estimation of the SPD lab standard deviation of measurement for known alcohol standards had been determined to be 1 for years 2005-July 2009. This was determined empirically from data gathered over the years and did not reflect a combined uncertainty as recommended by the ASCLD/LAB International guidelines.

b) The HSGC instruments to be used in the SPD lab were installed new in conjunction with the opening of the new facility. While the instruments were similar, they were not identical, as PerkinElmer had made upgrades in both software and firmware since the purchase of the original instrument.

c) Therefore, only data collected on the new instruments was used to calculate the uncertainty of measurement which is now to be reflected. Combined uncertainty was determined using the Root Sum Squares technique. The coverage factor was determined using the Student's t-table for $n \geq 100$ measurements to be 3.1 at a 99.7% confidence interval (CI). The combined uncertainty was determined to be 0.54. The expanded uncertainty is the combined uncertainty (U_c) x k and is equal to 1.6 for CI = 99.7. For a CI of 99.9999, k=6.1, and the U_c is 3.2.

d) Using a standard normal distribution, a CI of 99.9999 can be obtained by multiplying $U_{c\,(0.99)}$ by 5. As per the standard practice of the SPD lab,

that would yield a scientific certainty that the true value for any sample within ±5% of the measured value. This is consistent with the practice of our laboratory and well within the AZDPS regulatory requirement that obtained values for known alcohol samples be within ± 10% for a permit to be issued.

e) This uncertainty value may be reflected in a case file, in a report, or may be maintained in the uncertainty binder.

f) Uncertainty will be observed on an ongoing basis but recalculated only annually or when a significant change is made to the procedure or instrumentation.

VII. Appendices

VII.1. Abbreviations

Some abbreviations commonly used in the taking of blood alcohol notes are listed below:

item	abbreviation	meaning
a.	s	sealed
b.	m	marked
c.	mos	marked on seal
d.	rs	remedially sealed
e.	gtt	grey top tube
f.	pi	povidone-iodine
g.	P-I	povidone-iodine
h.	ttbk	tri-tech blood kit
i.	EE	evidence envelope
j.	T	taped
k.	T/S/M	tape-sealed and marked
l.	sm	small
m.	lg	large
n.	c	containing
o.	pl	plastic

VII.2 Revision History

Revision History – SPD Crime Lab Toxicology, Blood Alcohol Section Revision – description	
Table of contents. Space added between "other" and "liquids" for clarity section III.2.B	Modified by: JSV B1149 Date: 061510 Approved by: Kris Whitman Date: 6/22/10
Section III.2.D.5. The upper range of the calibration is changed in definition from ≥ 0.3 to ≥ 0.2 to encompass the upper statutory limit	Modified by: JSV B1149 Date: 061510 Approved by: Kris Whitman Date: 6/22/10
Section III.2.d.5. The phrase 'or equal to' was added to correct a clerical error.	Modified by: JSV B1149 Date: 080510 Approved by: Kris Whitman Date: 8/6/10
Section III.1.A.4. The phrase 'or equivalent' was added for flexibility.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section III.1.D.1. ~~and a copy will also be placed in the Disclosure binder under the appropriate date.~~ This phrase was removed to reflect that maintenance records are now being disclosed separately.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section III.1.D.3. The following phrases were added and removed to reflect the new requirement for noting the temperature and addressing what to do if the temperature check fails. The temperature ~~is to be noted as within or outside ("yes" or "no") the 1-5°C range on the~~ logsheets ~~which~~ are attached to the respective refrigerators and will be evaluated on a weekly basis. ~~This duty is to be performed by the analyst assigned to blood alcohol analysis for that week~~. These logs will be collected when complete and maintained in the appropriate BA maintenance logs binder. The temperature for the refrigerators will be maintained above freezing and below 8°C. If the temperature is outside of the acceptable range	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10

but is still cold, the analyst will adjust the temperature manually. If the appropriate range is still not attainable, the analyst will take the refrigerator out of service and move the samples or blood alcohol standards to another refrigerator within the lab which has an acceptable temperature as read on the thermometer. If the refrigerator is not cooling, it is to be immediately taken out of service and the blood or blood alcohol standards moved to a suitable refrigerator. Any time the refrigerator needs to be taken out of service, it will be recorded on the refrigerator log sheet.	
Section III.2.D.5. The phrase 'or equal to' was added for clarity.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section VI.1.I. The following phrase was added to address disputed discrepancies during technical review: Any discrepancies between the technical reviewer and the examiner which cannot be reconciled between themselves may be brought to the attention of the Technical Leader or the Quality Manager for evaluation of compliance with current standards and practices.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section VI.2.D. The following information was added and deleted to reflect the change in the name of the binder and how to address out of tolerance results. initials and date prepared will be maintained in the "Blood Alcohol "ISTD Verification" ~~of Blood Alcohol Calibrations and Standards~~ binder. If the internal standard does not meet this requirement, it will be discarded and reprepared. If there has been a significant change to the method or instrument such that this criteria cannot be met, it will be explained in the maintenance log and on the ISTD log.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section VI.2.F. The following wording was changed to reflect the name of the new binder: Validation information for the calibrators and ~~internal standards~~ will be maintained in the ~~"Verification of Blood Alcohol~~ "Calibrator ~~and Standards"~~ Verification" book.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section VI.3.C. : The following wording was changed to reflect the new binder name: Verifications of this validation will be maintained in the " QC Verification ~~of Blood Alcohol Calibrators and Standards~~" book.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10
Section VI.4.c. The wording for the uncertainty of measurement was changed as follows to reflect the new calculation and to correct a previous typographical error: The coverage factor was determined using the Student's t-table for n≥100	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman

measurements to be 3.1 at a 99.78% confidence interval (CI). The combined uncertainty was determined to be ~~1.00~~ 0.54. The expanded uncertainty~~, therefore,~~ is ~~given as~~ the ~~uncertainty~~ combined uncertainty (U_c) x k and is equal to ~~3.1~~ 1.6 for CI = 99.78. For a CI of 99.9999, k=6.1, and the U_c is 3.2.	11/16/10
Section III.1.D.2. Sentence was added for clarity. The pipettors will be calibrated annually on a schedule with the other pipettes by an outside vendor.	Modified by: JSV B1149 Date: 111510 Approved by: Kris Whitman Date: 11/16/10