

SCOTTSDALE POLICE DEPARTMENT FORENSIC SERVICES DIVISION-		
Toxicology		
Blood Alcohol Analysis Procedures Manual	Original Adoption Date: 2-22-10 Version: PM-TOX-001.1 Version Effective Date: 7-11-12 Issuing Authority: Kris Whitman, QAM	

**Toxicology Discipline Procedure Manuals
Blood Alcohol**

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1. Introduction

1.1. *Goals/objective/scope*

This is Scottsdale Police Department Crime Laboratory's procedure for the analysis of ethanol in biological fluids or other liquid matrices 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.

2. Personnel

2.1. Refer to the QSM for personnel specifications.

3. Evidence Control

3.1. This section will be used to address:

3.1.1. *Sample identification (unique number) /labeling*

All blood vials or other items of evidence for headspace gas chromatographic analysis submitted by the Scottsdale Police Department will have a unique item number assigned by the ILEADS system before reaching the laboratory. This number will be used to refer to specific test items and their results.

Blood vials or other items of evidence for headspace gas chromatographic analysis submitted by other agencies may not have a preassigned unique number for each item. If this is the case, the analyst will sub itemize the received item and give a unique number to the item(s) to be tested. This number will be used to refer to that specific item and its result(s).

3.1.2. *Chain of custody*

See QSM 5.8.1.1 for general chain of custody procedures. This applies to submissions from all agencies.

3.1.3. *Sample handling*

3.1.3.1. General. See QSM 5.8.4 for general sample handling procedures. In addition, all samples for analysis in the Toxicology section will be maintained in a refrigerated condition at any time that access is not required for testing.

3.1.3.2. Biological Sample Safety. 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.

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

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.

3.1.4. *Sample storage (long term and short term)*

Short term storage of test items in the Toxicology section may be in the refrigerator inside the evidence intake area or in the refrigerator inside the laboratory.

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For long term storage, items will be returned to Property and Evidence or the submitting agency.

- 3.1.5. *Sample security.* 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. Laboratory security is as outlined in QSM 5.3.

4. Validation

4.1. This section will be used to address:

4.1.1. *Method validation*

Only the established, validated method will be used for analysis for ethanol by headspace gas chromatography. Other volatiles may be added to the current method or the parameters may be changed to accommodate other volatiles. Refer to QSM 5.4

4.1.2. *How method/procedure modifications or variations during testing will be handled.*

4.1.2.1. Nonconforming work. There are times when the exact analytical protocol cannot be followed. In these cases the resultant casework is considered 'nonconforming' testing. Nonconforming testing is not inherently incorrect; it merely falls outside the bounds of the standard protocol. In the event that nonconforming testing is to be undertaken, it requires preapproval from the Toxicology Technical Leader or, in the absence of the Toxicology Technical Leader, the Quality Manager. A written memo explaining the nonconforming test proposal will be submitted and approved prior to the release of results for nonconforming work. Work itself may proceed on verbal approval from the Technical Leader or, in the absence of the Toxicology Technical Leader, the Quality Manager or their designee. An example of nonconforming work is when, in the opinion of the analyst, an insufficient sample volume is received for duplicate testing according to the regular sample preparation instructions. This can be addressed by performing a single test, manually pipetting the blood sample for replicates, or diluting the blood sample and analyzing replicates of the diluted sample. A copy of the approval memo will go in the case file and also be filed with the Quality Manager.

4.1.2.2. Minor method modifications. Minor method modifications may be required on an infrequent basis due to changes in instrument performance as equipment ages. These modifications may be made by the Technical Leader as necessary and the instrument will be tested with at least one set of standards prior to and after the modification to determine efficacy. If appropriate, the changes will be made to the SOP and a memo will be

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generated and signed by members of the section to indicate that they are aware of the modification(s) and will follow the amended protocol.

4.1.3. *Validation of new lots of testing kits*

4.1.3.1. Internally prepared standards

4.1.3.1.1 Calibrators. 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 $R^2 \geq 0.995$.

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 $\pm 3\%$ of their true values or 0.003 g/dL, whichever is greater, for the calibrators to be acceptable for use.

Calibrators may also be sent out to another crime laboratory for verification of accurate preparation.

Validation information for calibrators will be kept in the "Calibrator Verification" book.

4.1.3.1.2. Internal standard. Newly prepared internal standard (ISTD) solutions will be evaluated by preparing and analyzing a blank sample using 2500 μ l of the ISTD and demonstrating absence of any interfering compounds and an area count within $\pm 20\%$ 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 criterion cannot be met, it will be explained in the maintenance log and on the ISTD log.

4.1.3.1.3. Mixed standards.

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.

4.1.3.2. Externally acquired standards

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

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Analyzed concentrations must be within $\pm 3\%$ 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. Verifications of this validation will be maintained in the "QC Verification" book.

Externally acquired standards are supplied with an expiration date by the manufacturer. Exceeding this date does not necessarily render the standard unusable or invalid. If a standard is used past the expiration date it must be analyzed in the same manner it was initially validated (in duplicate against a valid calibration curve) as a closing verification when it is removed from use. This will be maintained in the "QC Verification" book along with the initial information. Standards which are removed from use prior to the manufacturer supplied expiration date are presumed to remain valid.

4.1.4. *Software upgrades, etc*

Software upgrades will be made only by a service representative. The new version of the software will be noted in the maintenance log and all other appropriate locations.

4.1.5. *Reference materials*

Covered above.

5. Analytical Procedures

5.1. This section will be used to address:

5.1.1. *Procedure/analytical method*

5.1.1.1 Blood.

- a. Remove one blood tube for analysis.
- b. Label two headspace vials with DR# (Departmental Record number) and subject's name and vial number.
- c. Before preparing each case sample check that the DR# and name on both vials correspond with DR# and name on blood tube.
- d. Dispense 2500 μ l n-propanol (0.015% w/v ISTD) and 250 μ l of sample from blood tube into headspace vial. Repeat for second vial. Cap and crimp both vials.
- e. Place prepared vials in rack in front of blood tube.

5.1.1.2. Other liquids.

- a. 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.
- b. Label two headspace vials with DR# and subject's name and vial number.

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- c. Before preparing each case sample check that the DR# and name on both vials correspond with DR# and name on sample tube.
- d. 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.
- e. Place prepared vials in rack.
- 5.1.1.3. Calibration Standards.
 - a. 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 13.1.1 and verified against known traceable standards or equivalent commercial standards as verified in 4.1.3.1.1.
 - b. Label headspace vials with calibration standard level.
 - c. Dispense 2500 µl n-propanol (ISTD) and 250 µl of calibration standard into headspace vial, cap and crimp.
- 5.1.1.4. Control Standards Preparation: Blank, Control Reference Material (CRM), Mixed and Check Standards preparation:
 - a. Prepare single vials for the mixed standard, CRMs, Check Standards, and blank in the same manner as samples and calibrators.
- 5.1.1.5. Place prepared vials into preassigned locations in the HS110 autosampler tray and continue analysis according to work instructions.
- 5.1.1.6. Capping. Capping of vials may be accomplished using a pneumatic crimper or a hand crimper. Despite the best efforts of the analyst, a cap may appear to be crimped properly but not be making a gastight seal. A loose cap may be identified post analysis as a sample having an internal standard area count more than 25% lower than the average internal standard area count measured for the controls analyzed in that batch for that analytical day.

5.1.2. *Sampling plan*

Not applicable.

Sampling Procedure

The sampling procedure for the analysis of whole blood or other liquid samples requires that the sample be homogeneous before removal of aliquots. This will be achieved by allowing all liquid samples to warm to room temperature, thoroughly mixing, and inspecting for clots. Clotted samples will be ground as necessary to homogenize.

Sample Selection

Any item for submitted for the same testing may be selected by the analyst either randomly or by evaluating volume or other physical properties which may make

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one item preferable to another. The item selected will be documented in the notes.

5.1.3. *Reagents*

Not applicable.

5.1.4. *Quantification*

5.1.4.1 Whole Blood: Prepare as described in the above and report as "...analysis of the blood..." and the lower of the two replicate values.

5.1.4.2. Clotted Blood: Place contents of collection tube into a tissue grinder and grind sample, then prepare as above. Report as "...analysis of the blood..." and the lower of the two replicate values.

5.1.4.3. 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 ethanol only. 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 (e.g. 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.

5.1.4.4. 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".

5.1.4.5. Report the lower of the two case results truncated to three decimal places for the valid duplicate.

5.1.4.6. 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 ..."

5.1.5. *Controls and Standards (traceability)*

NIST traceable standards are used whenever they are commercially available.

5.1.6. *Interpretation guidelines/uncertainty measurements*

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:

5.1.6.1 **Previous estimation.** 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

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empirically from data gathered over the years and did not reflect a combined uncertainty as recommended by the ASCLD/LAB International guidelines.

5.1.6.2 Current Instrumentation Initial Estimation.

5.1.6.2.1. Purpose. In 2009, 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.

5.1.6.2.2. Data. Only data collected on the new instruments was used to calculate the uncertainty of measurement which is now to be reflected. Quality control standards at all levels in whole blood matrix were used. 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 (U_e) is the combined uncertainty (U_c) x k ($k=3.2$) and is equal to 1.7 for CI = 99.7. For a CI of 99.9999, $k=5$, and the U_e is 4.95.

5.1.6.5 Calculation. Using a standard normal distribution, a CI of 99.9999 can be obtained by multiplying $U_c (0.54)$ 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 $\pm 5\%$ 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 $\pm 10\%$ for a permit to be issued.

5.1.6.6. Application. This uncertainty value may be reflected in a case file, in a report, or may be maintained in a file.

5.1.6.7. Reevaluation. Uncertainty will be observed on an ongoing basis but recalculated when a significant change is made to the procedure, instrumentation, or recommended method for calculation.

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6. Equipment Calibration and Maintenance

6.1 Equipment/Instruments used

6.1.1 Perkin-Elmer Instruments and software:

- 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.
- d. Quantitative method 'method2002A' or 'method2003A' for the A column and 'method2002B' or 'method2003B' for the B column or equivalent.

6.1.2. HP Laser Jet 4000 printer or comparable.

6.1.3 Dilutor/Dispenser: Hamilton Microlab 530B or comparable. Any calibrated pipette may be used for transfer of insufficient samples.

6.1.4 Capillary Columns:

Quantitative Column: PE Elite BAC 1, 30m x 0.32mm.

Confirmation Column: PE Elite BAC 2, 30m x 0.32mm.

6.1.5 Headspace Vials and Septa: 20 ml capacity with appropriate caps.

6.1.6 Mechanical or hand crimper for 20mm vials.

6.1.7 Ethanol: 200 proof anhydrous ethanol and externally prepared ethanol standards.

6.2 Performance checks

Incorporated in analytical method

6.3 Calibration if applicable

Incorporated in analytical method

6.4 Maintenance and schedule

6.4.1. 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.

6.4.2. 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 pipetter being taken from service and repaired. The pipettes will be calibrated annually on a schedule with the other pipettes by an outside vendor.

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6.4.3. 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.

6.5. Instrument method/conditions

6.5.1. Clarus 500 GC:

- 6.5.1.1 FID A and B:
 - a. Set H₂ to 45.0 ml/min flow.
 - b. Set Air to 450.0 ml/min flow.
- 6.5.1.2 GC conditions:
 - a. Detectors A and B: 250° C
 - b. Injector: 150° C
 - c. GC Oven: 38° C
 - d. Split Ratio: 10.0 ml/min
 - e. Run time: 4.00 min.

6.5.1.3 TurboMatrix 110 Autosampler:

- a. Needle: 70°C
- b. Transfer line: 80°C
- c. Vial Oven: 60°C
- d. Pressurization time: 1.0 min
- e. Injection time: 0.03 min
- f. Withdrawal time: 0.2 min
- g. Thermostat time: 22.0 min
- h. Cycle time: 4.0 min
- i. Column head pressure: 16 psi
- j. Inject Mode: Time
- k. HS Mode: Constant

6.5.2. Microlab 530B Diluter Pipetter parameters

- a. Left syringe size (µl): 2500
- b. Right syringe size (µl): 250
- c. Dilute method

Ratio 1: 10.0

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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
j. Syringe fill speed, left:	3
k. Syringe aspirate speed, right:	2
l. Syringe dispense speed, left:	4
m. Syringe dispense speed, right:	2
n. Syringe fill mode:	Auto
o. Air gap mode:	Auto
p. Air gap delay:	0.1
q. Wash volume (µl):	1250.0
r. Left fill speed:	3
s. Left dispense speed:	2

6.5.3. Analysis sequence parameters.

A standard sequence is analyzed in the following order:

Vial 1: 0.02% Calibration Standard

Vial 2: 0.10% Calibration Standard

Vial 3: 0.20% Calibration Standard

Vial 4: 0.40% Calibration Standard

Vial 5: Blank

Vial 6: Mixed Standard

Vial 7, 8, and last 2: Check standards as described above.

Vial 9, 10, et al: Duplicate Case Samples.

Middle: 2 or 4 mid-range check standards or CRMs.

Double check vial sequence in the TurboMatrix 110 tray before and after analysis.

7. Reports

7.1 This section will be used to address:

7.1.1. Case Documentation

7.1.1.1. *General note taking guidelines for specific unit*

Case notes for blood alcohol are taken directly in to JusticeTrax contemporaneously with the opening of the items. In the event that JusticeTrax is unavailable, notes may be taken by hand and later transferred into JusticeTrax by scanning in the handwritten notes and also transferring the data to the electronic sheet. This is necessary due to report generation requirements related to the electronic worksheet.

7.1.1.2. *How to generate notes (electronically or handwritten)*

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The blood alcohol section has a set worksheet to fill out. Additional information may be entered in the notes or comments areas of the worksheet.

7.1.1.3. *LIMS*

LIMS (JusticeTrax) is integral to the reporting of blood alcohol results. Much of the information that goes on the final report comes from the worksheet and the remainder comes from other pages in the JusticeTrax case file for each report. For specific information on how to use JusticeTrax for this purpose please refer to the work instructions.

7.1.1.4. *Note taking*

Refer to section 5.7/5.8. No additional information not covered in 7.1.1.1.

7.1.2. Reports

7.1.2.1. *Elements to include on the report*

Refer to QSM section 5.10.2.

7.1.2.2. *Recommendations for report writing*

None

7.1.2.3. *Testimony*

No additional information

7.1.3. Review

7.1.3.1. Qualifications of the technical reviewer. The technical reviewer must have been previously qualified to conduct blood alcohol analyses within a forensic laboratory through performing casework. Authorization to technically review cases in any specific section will be given and documented by the Quality Manager.

7.1.3.2. Elements of technical review. A second analyst must review and approve results of all analyses before results can be reported (100% technical review).

7.1.3.2.1. Technical review will consist of ensuring that the entire sequence including control and subject samples meet the criteria presented in appendix 13.2. The technical reviewer will signify that the sequence has met these criteria by initialing the face sheet for the sequence.

7.1.3.2.2. The technical reviewer will also ensure that all attachments to the file are identified with a unique identifier of either the DR number or the L number. The final report must contain the signature of the analyst, information about the analysts' permit, and about the disposition of the evidence after analysis is complete. The technical reviewer will also review all analysts' notes, results, and report for accuracy and signify acceptance by approving electronically in the LIMS system.

7.1.3.4. *Verification for positive identifications*

Not Applicable

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7.1.3.5. *Discrepancies*

Any discrepancies between the technical reviewer and the examiner which cannot be reconciled between them may be brought to the attention of the Technical Leader or the Quality Manager for evaluation of compliance with current standards and practices.

7.1.3.6. *Administrative Review*

The majority of the administrative review is performed during the technical review in this section. The remaining task is performed by the individual performing the administrative review and consists of finalizing the report by setting the milestone in LIMS and ensuring that the appropriate signatures and initials show on the final report. Administrative review may not be conducted by the author of the test report. Refer to QSM 5.9.5.

8. Safety

- 8.1 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.
- 8.2. All transferring of any potentially hazardous raw biological samples from one vial to another will be performed under a safety hood.
- 8.3. 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.
- 8.4. For additional information refer to SPD Lab Safety Manual PM-S-001.

9. Proficiency/Competency Testing

All analysts in the Toxicology Section performing blood alcohol analysis will be competency tested prior to beginning casework. All analysts will be proficiency tested on an annual basis in coordination with renewing their permits. Analysts performing headspace gas chromatographic analysis on nonbiological matrices will be competency tested prior to beginning casework but do not require a permit or an annual proficiency test.

See QSM Section 5.9.3 for further details.

10. Outsourcing

Not applicable.

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11. Glossary

Blank- is a vial containing Internal Standard.

CRM- (Control Reference Material) is a solution containing a known amount of ethanol obtained from an independent outside source. This may be a water or whole blood matrix.

Mixed Standard- 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.

Check Standards- 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 0.20.

Control Standards – Any ethanol and water or blood mixture of a known concentration which is used for the purpose of verifying the validity of the calibration curve.

ISTD (internal standard) – A mixture of water and a volatile in a known amount that is added to all samples and standards for purposes of dilution and normalization of data to variances in injections.

Calibration Standard – A standard of known concentration of ethanol and water which is used to establish the equation of calibration from which the unknown values will be calculated.

12. References

- Perkin Elmer Autosystem XL GC User's Guide © 1997 The Perkin-Elmer Corporation. Manual Part No. 0993-6073, Release D, December 1997.
- PerkinElmer TurboMatrix HS Control Software User's Guide © 2005 PerkinElmer, Inc. Manual Part Number 0993-06737, Release A, January 2005
- Perkin Elmer Turbochrom Workstation User's Guide. © 1989-1998 The Perkin-Elmer Corporation. Part Number S270-1601-B, May 1998
- PerkinElmer TotalChrom Workstation User's Guide © 2001 PerkinElmer Instruments LLC., Tidestone™ Formula One® Copyright 1993-2000 Tidestone Technologies, Inc. Portions Copyright © 1999 Blaise Software Services Inc. Portions Copyright © 1996 Microsoft Corporation. Part Number N515-6023, Release A, February 2001.

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Blood Alcohol Analysis Procedures Manual	Original Adoption Date: 2-22-10 Version: PM-TOX-001.1 Version Effective Date: 7-11-12 Issuing Authority: Kris Whitman, QAM	

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13. Appendices

13.1. Preparation of calibrators, mixed standard, and Internal standards.

13.1.1. Ethanol Calibration Standards:

13.1.1.1. SPD Crime Lab uses the following calibrators: 0.02, 0.10, 0.20, and 0.40% w/v ethanol in UltraPure water.

13.1.1.2. Prepare 500ml of 10.0% w/v stock solution using absolute ethanol and DIW at room temperature.

13.1.1.3. Using the 10% stock 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

13.1.2. 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

13.1.3. Internal Standard:

Prepare a stock solution of 15% w/v n-propanol (150 g/L) in UPW.

Prepare a working solution: 0.015% w/v (1ml stock to 1 liter).

Label all working and stock solutions with the contents, lot number, and date prepared.

13.1.4. Record all solution preparations in the appropriate log books.

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13.2 Analytical result quality assurance requirements.

- 13.2.1. Plot a calibration line. The calibration curve must have an R² 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 13.1.1.
- 13.2.2. The calibration curve will be valid for up to forty eight (48) hours.
- 13.2.3. 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.
- 13.2.4. 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.
 - 13.2.4.1. 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.
 - 13.2.4.2. 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 may be accepted as valid.
 - 13.2.4.2.1 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.
 - 13.2.4.2.2. 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.
 - 13.2.4.2.3. For loose caps it is permissible to uncap and recap a sample or quality control that has been determined to have a loose cap once it has cooled and to reanalyze that same vial. If the cap is still not sealing properly, the sample or CRM will need to be reprepared and analyzed according to section 5, above.
- 13.2.5. 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:
 - 13.2.5.1 Using the original calibration curve and analyzing newly prepared case samples at the end of the original sequence run followed by at least 2 calibration check samples, or
 - 13.2.5.2. Analyzing newly prepared case samples using a new calibration curve and controls.

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13.2.5.3. The results of all analyses will be recorded in the analysts' notes.

13.2.6. 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.

13.2.7. 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.

14. 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
p.	ctg	containing
q.	um	unmarked
r.	us	unsealed
s.	subj	subject
t.	SN	subject name
u.	mas	marked across seal
v.	init	initials
w.	ofc	officer
x.	coc	chain of custody

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15. Revision History

Revision History – SPD Crime Lab Toxicology, Blood Alcohol Section	
Revision – description	
Extensive revision of entire SOP undertaken to match the new format required by the SPD Crime Lab, and the newly formatted document carries a new version number. There are only 2 substantive changes. The first is that items q-x were added to the abbreviation table, which is now contained in section 14. The second is that section 12, the reference section, has been added and was not previously contained in the SOP. Table of contents. Space added between “other” and “liquids” for clarity section III.2.B	Modified by: Date: JSV B1149 010312
	Approved by: Date: MRaines B1466 042312