Blood Alcohol Analysis

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1. Introduction

1.1. The purpose of the Blood Alcohol Analysis Procedures Manual is to describe procedures 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 for confirmation.

2. Personnel

2.1. Personnel qualifications are addressed in the Quality Manual.

3. Evidence Control

3.1. Sample Identification (unique number)

All blood collection tubes 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 in the analyst's notes and report.

Blood collection tubes or other items of evidence for headspace gas chromatographic analysis submitted by agencies other than the Scottsdale Police Department may not have a pre-assigned unique number for each item. If this is the case, the analyst will sub-itemize the received item and give a unique number within the case to the item(s) to be tested. This number will be used to refer to that specific item and its result(s) in the analyst's notes and report.

3.2. Chain of Custody

Chain of custody procedures are addressed in the Quality Manual.

3.3. Sample Storage

General sample storage procedures are addressed in the Quality Manual. In addition, all samples for analysis in the Toxicology section will be maintained in a refrigerated condition whenever access is not required for part of the analysis process.

3.3.1. Short term sample storage

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 toxicology examination room.

3.3.2. Long term sample storage

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

3.4. 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 the evidence vault. If a visitor or service technician is present in an area that has unsecured evidence, samples must be attended or locked in a secure area.

4. Validation

The term validation is used in this manual to refer to the process of verifying that a method, reference material, solution, or test result is appropriate for its intended use.

4.1. Method Validation

Method validation is addressed in the Quality Manual

4.2. Reference Material Validation (Controls and Calibrators)

All externally acquired reference materials in water or whole blood matrix will be verified before use by analyzing in duplicate against the established calibration curve. Analyzed concentrations must be within ± 3% or 0.003 g/dl, whichever is greater, of the target value (supplied by the manufacturer) for the reference materials to be put in to use. Verifications of this validation will be maintained in the "QC Verification" book. Reference materials are presumed to remain valid until the manufacturer-supplied expiration date.

4.3. Internal Standard Solution Validation

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 re-prepared. 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 ISTD log.

4.4. Volatile Mixture (resolution test solution) Validation

The volatile mixture is used to establish qualitatively the ability of the method to separate a variety of volatile compounds 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 presence of a peak in the expected retention time window for each compound expected to be in the solution must be detected in the chromatogram of a newly prepared solution of the volatile mixture. If the volatile mixture does not meet this criterion, it will be discarded and re-prepared. The newly prepared solution may be verified for retention time against an externally prepared solution or the existing validated volatile mixture solution to check for the presence of all compounds.

4.5. Test Result Validation

Analysis of samples for ethanol is typically done as a batch process. Reporting a test result in a case requires acceptable batch quality assurance data and acceptable case specific quality assurance data for the case.

4.5.1. Batch Quality Assurance Data

4.5.1.1. Calibration Curve

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 be calculated based on the data points for the 0.02 g/dl, 0.10 g/dl, 0.20 g/dl, and 0.40 g/dl calibrators. The calibration curve will be valid for up to forty-eight hours.

4.5.1.2. Positive Controls

The measured ethanol concentration in all reference materials used as positive controls must be within $\pm$ 5% of the target value provided by the manufacturer. If one or more valid reference material test returns a value of greater than 5% outside the target value, the reason will be assessed.

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.

4.5.1.3. Blank

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.

4.5.1.4. Volatile Mixture

The chromatogram for the volatile mixture 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.

4.5.2. Case Specific Quality Assurance Data

4.5.2.1. Duplicate Test Agreement

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 using the original calibration curve and analyzing newly prepared case samples at the end of the original sequence run followed by at least 2 controls, or analyzing newly prepared case samples using a new calibration curve and controls. The results of all analyses will be recorded in the analysts' notes.

4.5.2.2. Ethanol Identification

The retention time of ethanol in case samples must be within 0.04 minutes of that of the calibrators for the batch. If this criterion is not met, the peak in the case sample may not be reported as ethanol. Ethanol must be identified on both columns in the analysis for ethanol to be reported.

5. Analytical Procedures

Analysis of liquid samples for ethanol content employs 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. Only the established, validated method will be used for analysis for ethanol by headspace gas chromatography.

5.1. 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. Homogeneity will be achieved by allowing all liquid samples to reach room temperature followed by thorough mixing. Blood samples will be visually inspected for clots. Clotted blood samples will be ground as necessary to homogenize prior to sampling. For serum or plasma samples, the supernatant will be separated from the cellular material prior to mixing the sample.

5.2. Sample Selection

If more than one blood collection tube or other container of liquid related to the same subject is submitted for ethanol testing in one case, either item may be selected by the analyst. The analyst may select the item either randomly, or by evaluating volume or other physical properties which may make one item preferable to another. The tube selected for analysis will be listed in the notes as tube #1 unless otherwise documented in the notes.

5.3. Deficient Samples

Submitted cases containing less than 2 milliliters of liquid will not be examined.

5.4. Sample Preparation.

Only one blood collection tube or other submitted container holding liquid for alcohol analysis will be open at the analyst's work area at any time. Samples will be transferred to 20-ml capacity headspace vials using the Microlab 530B Pipettor-Diluter, or comparable equipment and capped using a pneumatic crimper or a hand crimper with appropriate septa and caps. Despite the best efforts of the analyst, a cap may appear to be crimped properly but not have 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.

Prepared headspace vials will be placed into pre-assigned locations in the HS110 autosampler tray and the instrumental analysis will be started. The vial sequence in the TurboMatrix 110 tray should be checked before and after analysis.

5.4.1. Blood samples

The general procedure for removing blood from the collection tube and transferring the samples to headspace vials is outlined below. Serum and plasma samples will also be prepared in this manner.

a. Label two headspace vials with DR# (Departmental Record number) and subject's last name and vial number.

b. Remove one blood tube for analysis.

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 µl of the n-propanol internal standard solution and 250 µl 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.

5.4.2. Non-biological Liquids

The general procedure for removing non-biological liquids from the collection container and transferring the samples to headspace vials is outlined below

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 (or another reasonable factor) in purified water.

b. Label two headspace vials with DR# and subject's last 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 sample container.

d. Dispense 2500 µl of the n-propanol internal standard solution and 250 µl of sample from container into headspace vial. Repeat for second vial. Cap and crimp both vials

e. Place prepared vials in rack.

5.4.3. Calibrators

The general procedure for removing calibrators from their container and transferring the samples to headspace vials is outlined below

a. Use the validated aqueous 0.02, 0.10, 0.20 and 0.40 g/dl ethanol standards purchased from an outside vendor.

b. Label one headspace vial for each calibration level with the calibrator concentration and vial number.

c. Dispense 2500 µl of the n-propanol internal standard solution and 250 µl of calibrator into headspace vial, cap and crimp.

d. Place prepared vials in the rack.

5.4.4. Controls, Blank, and Volatile Mixture

Vials for the controls, blank, and volatile mixture will be prepared in the same manner as case samples and calibrators.

5.4.4.1. Controls

The controls are check standards at different concentrations. Each control sample will consist of 2500 µl n-propanol (ISTD) and 250 µl of the control. Eight control samples will be prepared, at least two of which will be whole blood controls. Two controls will be high-level controls (ethanol concentration ≥ 0.30 g/dl). Two controls will be low-level controls (ethanol concentrations ≤ 0.06 g/dl). Four controls will be mid-level controls (ethanol concentrations > 0.06 g/dl and < 0.30 g/dl). The controls will be distributed throughout the sequence such that one high and one low control are evaluated before any case samples and also after all case samples. In the approximate middle of the case samples two controls in the middle range will be evaluated along with the whole blood controls.

5.4.4.2. Blank

The blank serves as a negative ethanol control. The blank sample will consist of 2500 µl n-propanol (ISTD) and 250 µl of ultrapure water.

5.4.4.3. Volatile Mixture

The volatile mixture serves as a resolution test. The volatile mixture sample will consist of 2500 µl n-propanol (ISTD) and 250 µl of the volatile mixture.

5.5. Pipettor-Diluter Parameters

When preparing samples, the following parameters will be used on the pipettor-diluter.

Microlab 530B Pipettor-Diluter parameters

a. Left syringe size (µl): 2500
b. Right syringe size (µl): 250
c. Dilute method
    Ratio 1: 10.0
    Dilution 1: 11.0
    Left diluent volume (µl): 2500.0
d. Right air gap volume (µl): 5.0
    Right sample volume (µl): 250.0
    Final volume: 2750
e. Syringe fill speed, left: 3
f. Syringe aspirate speed, right: 2
g. Syringe dispense speed, left: 4
h. Syringe dispense speed, right: 2
i. Syringe fill mode: Auto
j. Air gap mode: Auto
k. Air gap delay: 0.1
l. Wash volume (µl): 1250.0
m. Left fill speed: 3
n. Left dispense speed: 2

5.6. Gas Chromatograph Parameters and Analysis Sequence

5.6.1. Gas Chromatograph Parameters

The gas chromatograph is equipped as follows:

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.
e. Capillary Columns:
   Quantitative Column: PE Elite BAC 1, 30m x 0.32mm.
   Confirmation Column: PE Elite BAC 2,30m x 0.32mm.

The gas chromatograph operating conditions are as follows:

a. Clarus 500 GC:

FID A and B:

Set H2 to 45.0 ml/min flow.

Set Air to 450.0 ml/min flow.

GC conditions:

Detectors A and B:	250° C
Injector:	150° C
GC Oven:	38° C
Split Ratio:	10.0 ml/min
Run time:	4.00 min.

b. TurboMatrix 110 Autosampler:

Needle:	70°C
Transfer line:	80°C
Vial Oven:	60°C
Pressurization time:	1.0 min
Injection time:	0.03 min
Withdrawal time:	0.2 min
Thermostat time:	22.0 min
Cycle time:	4.0 min
Column head pressure:	16 psi
Inject Mode:	Time
HS Mode:	Constant

5.6.2. Analysis sequence.

A standard sequence is analyzed in the following order:

Vial 1: 0.02 g/dl Calibrator

Vial 2: 0.10 g/dl Calibrator

Vial 3: 0.20 g/dl Calibrator

Vial 4: 0.40 g/dl Calibrator

Vial 5: Blank

Vial 6: Volatile Mixture

Vial 7, 8, and last 2: Each set will have one high-level control and one low-level control.

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

Middle: 4 mid-range controls. Two of the controls will be whole blood controls.

The vial sequence in the TurboMatrix 110 tray should be checked before and after analysis.

5.7. Handling Reference Materials

Reference materials will not be used past their provided expiration date. Directions for storage and use provided by the manufacturer will be followed when handling reference materials. For reference materials received in 1-ml ampoules, any remaining reference material will be discarded after that batch is prepared. For reference materials received in ampoules greater than 1 ml in size, the remainder may be transferred to an appropriate vial for storage and reuse.

5.8. Measurement Traceability

The traceability for this measurement process is established through the calibrators used to generate the calibration curve. The calibrators used for the analysis will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.

The equipment routinely used for this analysis that requires calibration from an external vendor is the pipettor-diluter. In addition, the analytical balances that are used as part of the internal performance check of the pipettor-diluter require calibration from an external vendor. The vendors who perform these calibrations will be accredited to ISO/IEC 17025:2005.

5.9. Measurement Assurance

Measurement assurance encompasses the practices put in place to monitor the testing process and to ensure the calibration status of equipment and reference materials used in the measurement process. These quality assurance procedures are documented throughout the protocol and are summarized in this section.

a. Analysts performing the tests are competency tested prior to beginning casework and complete an annual external proficiency test.

b. The method used for testing was validated prior to use in casework.

c. The gas chromatograph is maintained by an external technician.

d. The gas chromatograph is calibrated using external certified reference materials using a four-point calibration before cases are tested.

e. An internal standard is used in the testing process.

f. A blank sample is run.

g. Instrument resolution is tested using a volatile mixture.

h. Reference materials are tested to ensure that they are appropriate for their intended use prior to use and handled consistent with manufacturer's recommendations.

i. Reference materials purchased from external vendors in whole blood and aqueous matrices are used as controls. The blood matrix control is used as a quality assurance sample to guarantee that the method can accurately and adequately measure alcohol in a whole blood matrix.

j. Eight controls are included in each batch. These controls cover the high, middle, and low ends of the calibration curve.

k. Cases are tested in duplicate.

l. All cases go through technical and administrative review.

m. The pipettor-diluter used for preparation of samples and the balance used to performance check the pipettor-diluter are calibrated annually by an accredited external provider. The internal checks of the analytical balance by laboratory personnel are carried out using NIST-traceable weights.

n. The pipettor-diluter precision is checked internally on at least a quarterly basis.

5.10. Uncertainty of Measurement

A full explanation and information concerning the uncertainty of measurement is maintained as a separate document. The general outline is as follows:

Only data collected on the current instruments has been used to calculate the uncertainty of measurement. Quality control standards at all levels in whole blood and aqueous matrices were used. Using the Root Sum Squares technique, the combined uncertainty (Uc) was determined to be 1.1. Using the Student's t-table for $n \geq 100$ measurements, the coverage factor (k) is 3 at a 99.73% confidence interval (CI). To calculate the expanded uncertainty (Ue) the combined uncertainty (Uc = 1.1) is multiplied by the coverage factor (k=3) to arrive at 3.3 for a CI = 99.73. For a CI = 99.993, k = 4, so Ue is 4.4.

The statistical predictive value indicates that the true value of any test sample will be within 5% of the reported test value more than 99.993% of the time, or 99,993 times out of 100,000. The exact statistical prediction at 5% cannot be made at this time, however, a value of ± 5% will continue to be reported. 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.

The expanded uncertainty will be reported to at most two significant figures. The rounding method used for the expanded uncertainty is to always round up. The rounded expanded uncertainty will be reported to the same level of significance as the mean value of the duplicate test.

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.

6. Equipment Calibration and Maintenance

The equipment used in the section will be maintained and performance checked to ensure that it is operating properly.

6.1. Gas Chromatograph

6.1.1. Calibration

The instrument will be calibrated using external certified reference materials prior to casework analysis. The calibration curve will be calculated based on the data points generated for the 0.02 g/dl, 0.10 g/dl, 0.20 g/dl, and 0.40 g/dl calibrators. The calibration curve will be valid for up to forty-eight hours.

6.1.2. Maintenance and maintenance schedule

The Perkin-Elmer Clarus 500 and TurboMatrix 110 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.

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

6.2. Pipettor-Diluter Performance Checks

The performance of the pipettor-diluter will be checked internally and by an external vendor.

6.2.1. Precision Check

The pipettor-diluters will be assessed internally on a quarterly basis, or more frequently if needed. This assessment will consist of accessing the BA110 program on the control panel and weighing 10 aliquots of room temperature purified water and determining the mean and coefficient of variation 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 coefficient of variation must be less than or equal to 1 percent and the weights of each individual measurement must be within ± 0.5 percent of the mean weight for the ten measurements. Any check outside that will necessitate the pipettor-diluter being taken from service and repaired.

6.2.2. Calibration Check

The pipettor-diluters will have their calibration checked at least annually by an external vendor accredited to ISO/IEC 17025:2005.

6.3. Refrigerator Performance Checks

The Blood Alcohol section relies on refrigerators for the storage of standards and for the storage of subject samples in the laboratory. These refrigerators contain a NIST-traceable thermometer. The temperature logsheets are placed near the respective refrigerators and the temperature inside of the refrigerator 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.

7. Reports

This section addresses documentation of the testing process, how test results are to be reported, and the review process for test reports. Additional requirements for case documentation are found in the Quality Manual.

7.1. Case Documentation

Since most alcohol analyses are done as part of a batch, there is both case specific documentation and batch analysis documentation. Case specific documentation will be stored in the individual case files and batch data will be stored in a central location accessible to all examiners on the Y: drive.

7.1.1. Case Notes

Case notes for each blood alcohol cases are taken directly into JusticeTrax contemporaneously with the opening of the item. 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. The blood alcohol section has a set worksheet to fill out for each case. Additional information may be entered in the notes or comments areas of the worksheet.

7.1.2. Forensic Blood Alcohol Facesheet

A summary of the quality assurance data for each batch of samples will be recorded on the currently approved Forensic Blood Alcohol Facesheet. All of the appropriate data fields will be filled out on the Facesheet. In addition, a comment, such as, "All testing proceeded as expected." will be added under the "notes" section summarizing the run if no additional notation is required. Additional notation would be necessary if there was anything that affected the batch run, such as the automation not running to completion or concerns with any of the batch quality assurance data. Additionally, if anything happened during the run that resulted in the reanalysis of a case or hand correction of any computer generated data,documentation of the occurrence will appear on the Facesheet.

7.2. Case Results

Case results should be reported in a uniform manner to facilitate their interpretation by customers. For results that are reported as a numerical value, the mean of the duplicate test results will be reported along with the associated uncertainty in the test report. The mean will be determined using the measured values to the ten-thousandths place. The reported mean will be truncated to the thousandths place. The mean value will be reported with an expanded uncertainty that includes the coverage probability. The expanded uncertainty, calculated as the product of the truncated mean value and 0.05, will be reported to at most two significant figures. will be reported to at most two significant figures. The rounding method used for the expanded uncertainty is to always round up. The mean value and the rounded expanded uncertainty will be reported to the same level of significance.

The uncertainty of measurement on the test report will be reported as 5% at a level of confidence greater than 99.73%. This uncertainty of measurement has been administratively set and is greater than the actual calculated uncertainty of measurement at that level. The uncertainty will be reported in the same units of measure as that used to report the measured quantity value.

The final report must contain the signature of the analyst, information about the analyst's permit, and information about the disposition of the evidence after analysis is complete. Test reports documenting that no analysis was conducted do not require information about the analyst's permit

Additional requirements for reported information are addressed in the Quality Manual.

7.2.1. Whole Blood

Samples with a measured value greater than or equal 0.020 g/dl and less than or equal to 0.400 g/dl will be reported as outlined previously in this section. Samples with a measured value less than 0.020 g/dl and greater than or equal to 0.010 g/dl will be reported out as trace ethanol was detected or other similar phrasing. Samples with a measured value less than 0.010 g/dl will be reported as ethanol not detected. Samples with a measured value greater than 0.400 g/dl will be reported as greater than 0.400 or other similar phrasing.

7.2.2. Serum or Plasma

Serum or plasma samples may be reported as the sample is improper and no determination of blood alcohol concentration will be made. If the analyst chooses to analyze the sample, only the presence or absence of ethanol will be reported. Samples having a measured value less than 0.010 g/dl will be reported as ethanol was not detected in the serum or plasma. Samples having a measured value greater than or equal to 0.010 g/dl will be reported as ethanol was detected in the serum or plasma. 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 bold face capital letters) 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.

7.2.3. Non-biological Liquids

The results of the examination of a non-biological liquid for the presence of ethanol will be reported as ethanol was detected in the liquid or ethanol was not detected in the liquid. Samples having a measured value less than 0.010 g/dl will be reported as ethanol was not detected in the liquid. Samples having a measured value greater than or equal to 0.010 g/dl will be reported as ethanol was detected in the liquid.

7.2.4. Deficient samples

Submitted cases containing less than 2 milliliters of liquid will not be examined. The test report for such cases will indicate that the item was not tested because the item contained insufficient sample size to be tested using the Laboratory's current protocol.

7.3. Case Review

All cases will undergo technical and administrative review before a report is issued.

7.3.1. Technical Review

Technical review will only be done by qualified approved staff.

7.3.1.1. 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.

7.3.1.2. Elements of technical review.

The first phase of technical review will consist of ensuring that the batch quality assurance data meet quality expectations that have been established in this manual to indicate that the instrument was functioning properly during the testing process. The technical reviewer will signify that the batch quality assurance data has met the criteria presented in this manual by initialing the face sheet for the sequence.

The second phase of technical review will consist of ensuring that the case specific quality assurance data meet the expectations established in this manual. Each case is reviewed individually. The technical reviewer will signify that both the batch quality assurance data and the case quality assurance data meet the criteria presented in this manual by setting the milestone in JusticeTrax resulting in the application of the reviewer's initials on the test report. The technical reviewer will also ensure that all attachments to the case file are identified with a unique identifier of either the DR number or the L number. The technical reviewer will also review the analyst's notes, results, and report for accuracy.

7.3.1.3. 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.

7.3.2. 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 JusticeTrax 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. Administrative Review is also addressed in the Quality Manual.

8. Safety

General laboratory safety procedures are addressed in the Laboratory Safety Manual.

The following section specific safety procedures should be followed when handling potentially infectious material:

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.

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.

Proficiency and competency testing are further addressed in the Quality Manual.

10. Outsourcing

Outsourcing is addressed in the Quality Manual.

11. Glossary

***Calibrator* – (**VIM 5.12) measurement standard used in calibration.
***Calibration Curve* -** (VIM 4.31) - expression of the relation between indication and corresponding quantity value.
***Blank* -** An ethanol-free sample (also known as a negative control).
***Certified Reference Material* - (**VIM 5.14) Reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.
***Reference material* -** Material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.
***Control (positive)* –** 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.

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.
- PerkinElmer TurboMatrix Headspace Sampler and HS 40/110 Trap User's Guide © 2005 PerkinElmer, Inc. Release M04103401 E November 2005.

- Hamilton Microlab ® 500B/C Series User's Manual © 1996 by Hamilton Company. Part Number 69176 (Rev. C)
- Joint Committee for Guides in Metrology (JCGM), International vocabulary of metrology – Basic and general concepts and associated terms (VIM), 3rd ed. (Serves, France: International Bureau of Weights and Measures [BIPM]-JCGM 200, 2012) (2008 with monor corrections).

13. Appendices

13.1. Solution Preparation.

Preparation of all solutions will be recorded in the appropriate log book. Label all working and stock solutions with the contents, lot number, and date prepared. Solutions may be prepared in volumes other than those listed in this appendix.

13.1.1. Internal Standard Solution

The concentration of the internal standard solutions is not critical for analytical accuracy. Therefore, the concentration only needs to be approximate.

13.1.1.1. Internal standard stock solution

Prepare 100 ml of a 15% w/v n-propanol stock solution by transferring 15 grams of n-propanol into a 100-ml volumetric flask partially filled with ultrapure water. Dilute the mixture in the volumetric flask to the line with ultrapure water. Mix the solution.

13.1.1.2. Internal standard working solution

Prepare 4 L of 0.015% w/v working internal standard solution by pipetting 4 ml of the 15% w/v n-propanol stock solution into approximately 4 L of ultrapure water. Mix the solution. Validate the solution using the procedure outlined in this manual.

13.1.2. Volatile Mixture (resolution test solution)

The concentration of the volatile compounds in the volatile mixture is not critical. Therefore, the concentrations only need to be approximate.

The volatile mixture consists of 0.02% w/v acetaldehyde, 0.02% w/v acetone, 0.08% w/v isopropyl alcohol, 0.08% w/v methanol, and 0.079% w/v ethanol. Prepare the volatile mixture by pipetting the following volumes into a 1000-ml volumetric flask partially filled with ultrapure water:

Chemical	(ml)
Acetaldehyde	0.25
Acetone	0.25
Ethanol	1.0
Isopropyl alcohol	1.0
Methanol	1.0

Note: Acetaldehyde must be pipetted while the acetaldehyde is cold.

Dilute the mixture in the volumetric flask to the line with ultrapure water. Validate the solution using the procedure outlined in this manual.

13.2. Corrective Quality Control and Non-conforming Work

This appendix addresses how to handle the infrequent occurrences when testing does not meet the quality requirements specified in this document or when testing cannot proceed using the specified protocol.

13.2.1 Corrective Quality Assurance

The quality assurance program put into place per this manual is very effective at ensuring the overall quality of reported test results. This section of the manual addresses how to handle some instances in which the quality criteria are not met. Issues not addressed in this section will be addressed on a case by case basis. Whenever possible, the root cause of the issue should be identified and addressed.

- Issue: Ethanol is detected in the blank sample.
  Correction: Identify and use an ethanol-free blank and reanalyze the entire batch.

- Issue: One or more control is outside of the $\pm$ 5% target range.
  Correction: Identify and use a valid control and reanalyze the entire batch.

- Issue: The calibration curve has an R^2 less than 0.995.

Correction: identify and use a valid set of calibrators and reanalyze the entire batch.

- Issue: Duplicate case samples do not agree within 5% or 0.005 g/dl, whichever is greater.
  Correction: Analyze the case samples again by either using the original calibration curve and analyzing newly prepared case samples at the end of the original sequence run followed by at least 2 controls, or analyzing newly prepared case samples using a new calibration curve and controls.

13.2.1. Non-conforming 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. A copy of the approval memo will go in the case file and also be filed with the Quality Manager.

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

14. Abbreviations

Abbreviations commonly used when recording blood alcohol notes are listed in this section. Abbreviations may appear as capital or lower-case and with or without periods when used. However, the abbreviations IS and US, which spell actual words, must appear as capital letters when used.

Abbreviation number	Abbreviation	Meaning
1	b#	badge number
2	bzk	benzalkonium chloride
3	c / ctg	containing
4	coc	chain of custody
5	ee	evidence envelope
6	gtt	grey top tube
7	init	initials
8	istd / IS	internal standard
9	lg	large
10	los	label over seal
11	m	marked
12	mas	marked across seal
13	mos	marked on seal
14	ofc	officer
15	pi / p-i	povidone-iodine
16	pl	plastic
17	rs	remedially sealed
18	s	sealed
19	sm	small
20	subj	subject
21	sn	subject name
22	t	taped
23	ttbk	tri-tech blood kit
24	um	unmarked
25	US	unsealed
26	ns	Not sealed
27	parens	parenthesis
28		
29		

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: JSV B1149 Date: 010312 Approved by: MRaines B1466 Date: 042312
Clarified section on unique numbering. Replaced all references to specific sections in the Quality Manual. Changed sample handling section to sample storage and moved biological safety portion to the safety section of the manual. Revised validation section to reflect verification process. Removed method modification section from validation and placed it into an appendix. Removed all references to internally prepared calibrators. Replaced references to mixed standard with volatile mixture. Added test result validation to validation section. Changed requirements for controls to be ±5% throughout the range of controls. No longer acceptable to use expired controls. Specified that ethanol must be detected on both columns to report as ethanol. Revised analytical procedures section. Added section on deficient samples. Specified that the blank will be internal standard solution and water. Added section on handling reference materials. Added section on Measurement traceability. Added section on measurement assurance. Updated information in the uncertainty of measurement section. Reformatted equipment and calibration section. Expanded Reports section. Added	Modified by: PAK B1255 Date: 11/20/13 Approved by: Mraines B1466 Date: 12/9/13

storage of batch data. Required use of Facesheet and specified what to document on Facesheet. Specified how uncertainty will be reported. Specified that serum, plasma, non-biological liquids will be reported as ethanol detected or not detected. Specified that measured results > 0.400 would be reported as > 0.400. Replaced all words in the glossary. Added one reference. Expanded instructions for solution preparation. Replaced appendix 13.2 with a corrective quality control and non-conforming work section. Reformatted abbreviations section and added abbreviations. Added section on reporting insufficient samples.	
The revisions involve changing > 0.30 to ≥ 0.30 and < 0.06 to ≤ 0.06 in 5.4.4.1 with reference to high-level controls and low-level controls, respectively. In addition, it was made clear in section 5.4 that only one blood tube was to be open at a time. In section 7.2.1, d/dl was changed to g/dl. A statement was added to section 7.2 specifying that the uncertainty is calculated as the product of the truncated mean value and 0.05. The pipettor-diluter precision check was changed from using the MAINT program to the BA110 program. The parameters for passing the precision check were changed from a standard deviation less than 0.033 to a coefficient of variation of less than or equal to 1% and each of the recorded weights must be within ± 0.5 percent of the mean weight for the ten measurements.	Modified by: Date: PAK B1255 03/06/14 Approved by: Date: Mraines B1466 03/11/14
