

**SCOTTSDALE POLICE DEPARTMENT FORENSIC LABORATORY**

**Title: Blood Alcohol Procedures Manual**

**Document ID: 1084**



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**Issuing Authority: Allan Kosecki, Discipline Technical Leader**

**Blood Alcohol Analysis**

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## **1. Introduction**

The purpose of the Blood Alcohol Procedures Manual is to describe procedures for the analysis of ethanol in whole blood 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**

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 submitted by the Scottsdale Police Department for headspace gas chromatographic analysis will have a unique item number assigned by the records management 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 submitted by agencies other than the Scottsdale Police Department for headspace gas chromatographic analysis 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 in the custody of an analyst 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 will be in the refrigerator inside the toxicology examination room.

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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. 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/Verification**

The term verification 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 Verification (Controls and Calibrators)

All externally acquired reference materials in water or whole blood matrix will be verified before use by analyzing in duplicate in a run incorporating all the quality assurance measures outlined in this manual. 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 reference materials to be put in to use. Documentation of this verification will be maintained by the laboratory. Reference materials are presumed to remain valid until the manufacturer-supplied expiration date. Whole blood used as a negative control will be evaluated prior to use by adding 0.25 mL of the blood to 2.5 mL of deionized water in a headspace vial and analyzing the sample using the ethanol quant method on the gas chromatograph. The chromatogram from the blood sample must not have any peaks that would interfere with the quantitation of ethanol. Documentation of this verification will be maintained by the laboratory.

4.3. Internal Standard Solution Verification

Newly prepared internal standard (ISTD) solutions will be evaluated by preparing and analyzing a blank sample using 2500  $\mu\text{l}$  of the ISTD and 250  $\mu\text{l}$  of water and demonstrating absence of any interfering compounds and an n-propanol area count

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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 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) Verification

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.

#### 4.5. Test Result Verification

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  $\geq 0.995$  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, case results from the analytical run will not be reported.

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The retention time of ethanol in a chromatogram from a batch must be within 0.02 minutes of that of the calibration standards for the batch. If this criterion is not met the peak may not be reported as ethanol. The area counts for the internal standard must be within 25% of the mean internal standard area counts for the calibrators.

**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. The area counts for the internal standard must be within 25% of the mean internal standard area counts for the calibrators.

**4.5.1.4. Volatile Mixture**

The chromatogram for the volatile mixture in a run must show separation of the five components and the internal standard. 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. The area counts for the internal standard must be within 25% of the mean internal standard area counts for the calibrators.

**4.5.2. Case Specific Quality Assurance Data**

**4.5.2.1. Duplicate Test Agreement**

Results of duplicate case samples must be within 2% or 0.0020 g/dL, whichever is greater, of the mean of the two results. If sample duplicates are not within tolerance, then the case sample will be analyzed again by 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 and Volatiles Identification**

The retention time of ethanol in case samples must be within 0.02 minutes of that of the calibrators for the batch. If this criterion is not met, the peak

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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. Volatiles other than ethanol must be detected on both columns within 0.03 minutes of their retention time in the volatiles mix for the notes to reflect that the volatile was detected.

**4.5.2.3 Internal Standard Area Counts**

The area counts for the internal standard must be within 25% of the mean internal standard area counts for the calibrators.

**5. Analytical Procedures**

Analysis of blood 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 an established, validated method will be used for analysis for ethanol by headspace gas chromatography. All testing will be done in the controlled environment of the limited access toxicology lab.

**5.1. Deficient Samples**

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

**5.2. Sample Selection**

If more than one blood collection tube 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.

**5.3. Sample Types**

The samples in a batch include quality assurance samples, case samples, and occasionally other samples (e.g. proficiency samples or controls requiring verification).

**5.3.1. Calibrators**

The calibrators are aqueous solutions purchased from an outside vendor having known ethanol concentrations. The following calibration levels will be used: 0.02, 0.10, 0.20 and 0.40 g/dL ethanol. The calibrators will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.

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**5.3.2. Controls**

The controls are solutions containing ethanol at different concentrations either in water or blood. At least ten percent of the samples in a batch will be positive controls and at least two control samples will be whole blood controls. Two controls will be high-level controls (ethanol concentration  $\geq 0.30$  g/dL). Two controls will be low-level controls (ethanol concentrations  $\leq 0.06$  g/dL). The remaining 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-level control, one low-level control, and one mid-level control are evaluated before any case samples and also after all case samples. At least one of these pre-case and post-case controls will be a whole blood control. Controls will be placed in a batch such that every five cases are bracketed by a control. At least one control will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.

**5.3.3. Blank (negative control)**

A blank serves as a negative ethanol control. A blank sample will consist of 2500  $\mu$ l n-propanol (ISTD) and 250  $\mu$ l of ultrapure water or ethanol free blood. A blank will be analyzed near the beginning of a batch and at the end of a batch.

**5.3.4. Volatile mixture**

The volatile mixture serves as a resolution test. The volatile mixture contains acetaldehyde, methanol, ethanol, isopropanol, and acetone.

**5.3.5. Biological case samples**

Case samples will be analyzed in duplicate.

**5.4. Sample Preparation**

The sampling procedure for the analysis of whole blood requires that the sample be homogeneous before removal of aliquots. Homogeneity will be achieved by thorough mixing. Blood samples will be visually inspected for clots. Clotted blood samples will be ground as necessary to homogenize prior to sampling. The quality assurance and case samples should also be approximately the same temperature when sampled. Accordingly, all material to be used for a batch should be removed from the refrigerator at the same approximate time and at least one hour prior to sampling.

**5.4.1. Headspace vial preparation**

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Headspace vials will be labeled for each sample that will be analyzed. For each case sample, label two headspace vials with DR# (Departmental Record number) or Lab number and subject's last name and vial number. For each quality assurance sample, label one headspace vial with the sample name and vial number. The labeled headspace vials should be placed in racks according to vial number.

**5.4.2. Kit examination**

Kits will be examined one at a time and notes as described in section 7.1.1 will be taken contemporaneously. As part of this process, one tube from each case will be selected for analysis.

**5.4.3. Sample pipetting**

Only one blood collection tube for alcohol analysis will be open at the analyst's work area at any time. Before preparing each case sample, check that the case identifying information on both vials corresponds with the case identifying information on the blood tube. Before preparing each quality assurance sample, check that the sample identity on the headspace vial matches that on the solution container. Using the pipette diluter, 2500  $\mu$ l of the n-propanol internal standard solution and 250  $\mu$ l of the sample will be dispensed into a 20-mL headspace vial. The headspace vial will then be capped and crimped using a pneumatic or hand-held crimper. The prepared vials will be returned to their location in the rack prior to preparation of the next samples. Prior to loading the headspace vials on the autosampler, the vials should be gently swirled to ensure homogeneity of the sample and internal standard mixture within the headspace vial. Prepared headspace vials will be placed into pre-assigned locations on the auto sampler. The vial sequence on the auto sampler should be checked before and after analysis. Samples should be pipetted in the order in which they will be analyzed on the instrument except if multiple samples of aqueous control will be pipetted from a single ampoule.

Each headspace vial will contain 250  $\mu$ l of the sample and 2500  $\mu$ l of the n-propanol internal standard solution. The same lot of internal standard that was used for the calibrators will be used for all samples analyzed based on those calibrators.

Samples will be prepared using method BA 250 to 2500 on the Microlab series 600.

**5.5. Analysis Sequence**

A standard sequence containing 45 cases is analyzed on the gas chromatograph in the following order:



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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 and last vial: Blank

Vial 6: Volatile Mixture

Vial 7, 8, 9, and last 3 vials before ending blank: Each set will have one high-level control, one low-level control, and one mid-level control.

Vial 10-19, 21-30, 32-41, 43-52, 54-63, 65-74, 76-85, 87-96, and 98-107 duplicate case samples.

Vials 20, 31, 42, 53, 64, 75, 86, and 97 mid-level controls.

Adjustments will be made for batches containing less than the 45 cases.

## 5.6. Gas Chromatograph Parameters

### 5.6.1. Agilent Gas Chromatograph Parameters

#### 5.6.2. The gas chromatograph is equipped as follows:

- a. Model 7890B serial number US14173023.
- b. Headspace sampler 7697A serial number CN14160045.
- c. OpenLAB software version C.01.06 or higher.
- d. Quantitative method 'ethanol quant'.
- e. Capillary Columns:

Quantitative Column: DB-ALC1 30 m x 320 µm x 1.8 µm

Qualitative Column: DB-ALC2 30 m x 320 µm x 1.8 µm

#### 5.6.3. The gas chromatograph operating conditions are as follows:

##### 7890B gas chromatograph

GC oven	40° C
GC run time	2.8 minutes
GC cycle time	4.0 minutes
Carrier gas	hydrogen
Front inlet temperature	110° C
Pressure	10 PSI
Septum purge flow	3 ml/min
Split ratio	10:1
FID temperature	300° C
FID hydrogen flow	30 ml/min
FID air flow	400 ml/min

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FID nitrogen flow      25 ml/min

7697A headspace sampler

Vial pressurization gas      nitrogen

Loop size                  1 ml

Oven temperature          60° C

Loop temperature          60° C

Transfer line temperature 90° C

Vial equilibration          22 minutes

Injection duration          0.5minutes

Vial fill pressure              15 psi

Loop ramp rate                30 psi/min

Loop final pressure      1.5 psi

Loop equilibration time      0.05 minutes

5.6.4 The calibration model is a linear regression based on the four calibrators used. The curve fit is not forced through the origin.

**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 pipette diluter. In addition, the analytical balances that are used as part of the internal performance check of the pipette diluter require calibration from an external vendor. The vendors who perform these calibrations will be accredited to ISO/IEC 17025.



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### 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. At least two blank samples are run.
- g. Instrument resolution is tested using a volatiles 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 confirm that the method can accurately and adequately measure alcohol in a whole blood matrix. Aqueous reference materials must be from a different vendor or lot number than the calibrators. At least one control will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.
- j. At least ten percent of the samples in a batch are controls. These controls cover the high, middle, and low ends of the calibration curve.

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- k. Cases are tested in duplicate.
- l. All cases go through technical and administrative review.
- m. The pipette diluter used for preparation of samples and the balance used to performance check the pipette diluter are calibrated annually by an accredited external provider. The internal checks of the analytical balance by laboratory personnel are carried out using calibration weights that are calibrated by an external vendor accredited to ISO/IEC 17025.
- n. The pipette 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 in whole blood were used to assess measurement process reproducibility. Using the Root Sum Squares technique, the combined uncertainty ( $U_c$ ) was determined to be  $< 1.6\%$ . Using the Student's  $t$ -table for  $n \geq 100$  measurements, the coverage factor ( $k$ ) is 3 at a 99.73% confidence level. To calculate the expanded uncertainty ( $U_e$ ) the combined uncertainty was multiplied by the coverage factor ( $k=3$ ) to arrive at an expanded uncertainty  $< 5\%$  with a coverage probability of 99.73%. Therefore, the interval created by multiplying the measured blood alcohol concentration by  $\pm 5\%$  contains the sample's true blood alcohol concentration at least 99.73% of the time.

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 at least once per year. If the expanded uncertainty remains less than 5%, no changes will be in the reporting methodology.

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## **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 gas chromatographs will be maintained on a preventative basis by a qualified vendor. Any repairs or maintenance required outside of the regular schedule will be performed as needed by a qualified vendor. All repairs and maintenance records will be kept by the Lab. A sample of the volatile mixture will be injected following any maintenance on the gas chromatograph. The resulting chromatogram will be analyzed for acceptable resolution and peak shape prior to returning the instrument to service.

6.1.3. 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. Pipette Diluter Performance Checks**

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

#### **6.2.1. Precision Check**

The pipette diluters will be assessed internally on a quarterly basis, or more frequently if needed. This assessment will consist of accessing the method used for casework 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 by the lab. 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  $\pm 0.5$  percent of the mean weight for the ten measurements. Any check outside that will necessitate the pipette diluter being taken from service and repaired.



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#### 6.2.2. Calibration Check

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

#### 6.3. Refrigerator Performance Checks

The Toxicology section relies on refrigerators for the storage of reference materials, solutions, and subject samples in the laboratory. The temperature inside of these refrigerators will be continuously monitored using an automated recording/monitoring system. The temperature for the refrigerators will be maintained above freezing and below 8°C.

#### 6.4. Balance Calibration Verification

The calibration status of the balance will be verified less than seven days prior to being used to perform a precision check on the pipette dilutor. The verification will be conducted using calibration weights calibrated by an external vendor accredited to ISO/IEC 17025. The results of these checks will be recorded in the balance verification logbook. The balance should be clean and free of debris prior to the calibration verification process. The balance pan may be cleaned with a damp towel. The balance will be checked to ensure that it is level prior to use. The enclosure around the pan, the door to the enclosure will be closed prior to taring the balance or allowing the balance to stabilize as part of recording the weight.

The balance will be checked at 1 gram and at 200 grams. The check at 1 gram must be within +/- 2 milligrams and the check at 200 grams must be within the +/- 20 milligrams or the balance will be re-calibrated using ANSI/ASTM Class I weights, the balance's internal calibration procedure, or removed from service and repaired.

### 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

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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 case 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. Completion of the electronic worksheet 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. Blood Alcohol Facesheet**

A summary of the quality assurance data for each batch of samples will be recorded on the currently approved 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. Correction of minor typographical errors in a subject's name are not required to be documented 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. 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.

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The uncertainty of measurement on the test report will be reported as 5% at a coverage probability 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 g/dL or other similar phrasing.

#### 7.2.2. Deficient samples

Submitted samples 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.

#### 7.2.3. Plasma/Serum samples

Blood tubes containing plasma or serum will not be analyzed. The test report for such cases will indicate that the item was not tested because the Laboratory's current protocol only permits analysis of whole blood samples.

### 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.



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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 as specified in the Quality manual.

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/signing 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 Lab 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

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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.

8.1. 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 handling of an open container of any potentially infectious biological material will be performed in a biosafety cabinet.

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).

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***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

- 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 minor corrections).
- Microlab ® 600 Basic Manual © 2013 by Hamilton Company. Part Number 61440-01 (Rev. F).
- Microlab ® 600 Advanced Manual © 2013 by Hamilton Company. Part Number 61440-01 (Rev. D).
- Agilent 7697A Headspace Sampler Advanced Operation Agilent Technologies, Inc. 2011.
- Agilent 7697A Headspace Sampler Operation Agilent Technologies, Inc. 2011.

## 13. Appendices

### 13.1. Solution Preparation and Storage.

Preparation of all solutions will be recorded in the appropriate logbook. Label all working and stock solutions with the identity of the reagent and date of preparation or lot number. 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

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Prepare 50 ml of a 15% w/v n-propanol stock solution by transferring 7.5 grams of n-propanol into a 50-ml volumetric flask partially filled with ultrapure water. Dilute the mixture in the volumetric flask to the line with ultrapure water. Mix the solution. The solution will expire two years after preparation. The solution will be stored in a refrigerator.

13.1.1.2. Internal standard working solution

Prepare 5 L of 0.015% w/v working internal standard solution by pipetting 5 ml of the 15% w/v n-propanol stock solution into approximately 5 L of ultrapure water. Mix the solution. Validate the solution using the procedure outlined in this manual. The solution will have the same expiration date as that of the stock solution from which it was made.

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 0.08% w/v ethanol. Prepare the volatile mixture by pipetting the following volumes into a 100-ml volumetric flask partially filled with ultrapure water:

<u>Chemical</u>	<u>(<math>\mu</math>l)</u>
Acetaldehyde	25
Acetone	25
Ethanol	100
Isopropyl alcohol	100
Methanol	100

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. The solution will be stored in the refrigerator.

13.2. Corrective Quality Control and Non-conforming Work

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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 2% or 0.0020 g/dL, whichever is greater, of the mean of the two results.  
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.
- Issue: Internal standard area counts that are more than 25% less than or greater than the mean internal standard area count for the calibrators in the run.  
Correction: The sample is not a valid analysis. Corrective action varies depending on if the sample is a case sample or quality assurance sample.

#### 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

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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 be maintained by the laboratory.

**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 procedures manual and members of the section will be made aware of the modification(s) and instructed to follow the amended protocol.

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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	exp	expiration
2	bzk	benzalkonium chloride
3	c / ctg	containing
4	coc	chain of custody
5	ee	evidence envelope
6	DR	department report or department record
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/po	Officer/police 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	rna	received not analyzed
24	um	unmarked
25	US	unsealed
26	ns	Not sealed
27	parens	parenthesis
28	SPD	Scottsdale Police Department
29	SRPD	Salt River Police Department
30	pA	picoamp
31	L	liter
32	Mls or ml	Milliliter(s)

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Abbreviation number	Abbreviation	Meaning
33	min	minute
34	FID	Flame ionization detector
35	LIMS	Laboratory information management system
36	g	gram
37	w/v	Weight per volume
38	evid	evidence
39	dL	deciliter
40	μL	microliter
41	ISTD	Internal standard



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**15. Revision History**

Revision History – SPD Crime Lab Toxicology, Blood Alcohol Section		
Revision – description		
<p>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</p>	Modified by: JSV B1149	Date: 010312
	Approved by: MRaines B1466	Date: 042312
<p>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 <math>\pm 5\%</math> 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</p>	Modified by: PAK B1255	Date: 11/20/13
	Approved by: Mraines B1466	Date: 12/9/13

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<p>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 &gt; 0.400 would be reported as &gt; 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.</p>					
<p>The revisions involve changing &gt; 0.30 to <math>\geq 0.30</math> and &lt; 0.06 to <math>\leq 0.06</math> 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 <math>\pm 0.5</math> percent of the mean weight for the ten measurements.</p>	<table border="1"> <tr> <td>Modified by: PAK B1255</td> <td>Date: 03/06/14</td> </tr> <tr> <td>Approved by: Mraines B1466</td> <td>Date: 03/11/14</td> </tr> </table>	Modified by: PAK B1255	Date: 03/06/14	Approved by: Mraines B1466	Date: 03/11/14
Modified by: PAK B1255	Date: 03/06/14				
Approved by: Mraines B1466	Date: 03/11/14				
<p>Changed short term storage location. Changed requirements for duplicate test agreement. Extensive reorganizing of the analytical procedures section. Added recommendation that all batch material be removed from refrigerator at the same time. Moved loose cap data to corrective quality assurance section. Changed placement of controls within the run. Added an additional blank</p>	<table border="1"> <tr> <td>Modified by: PAK B1255</td> <td>Date: 08/05/15</td> </tr> </table>	Modified by: PAK B1255	Date: 08/05/15		
Modified by: PAK B1255	Date: 08/05/15				

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<p>sample. Removed use of refrigerator in evidence vault and changed temperature checking requirements. Volumes for some solutions were changed. Added Agilent instrument. Specified ISTD validation samples include 250 µl of water. Changed requirements for reference material validation from run against established calibration curve to run in a batch incorporating all quality assurance procedures outlined in this manual. Added a second blank at the end of the run. Added recommendation that filled headspace vials be swirled gently prior to loading on autosampler. Removed instrument 650N9042003. Added expiration dates for the internal standard stock solution and working solution. Added storage conditions for the internal standard stock solution, working solution, and volatile mixture. Changed TR authorization from QM to FLM.</p>	
<p>Changed Corrective Quality Assurance Section to reflect current requirement for duplicate test agreement.</p>	<p>Modified by: PAK B1255      Date: 08/27/15</p>
<p>4.5.1.2 and 4.5.2.2. changed the retention time requirement from 0.04 minutes to 0.02 minutes. 5.4.3. replaced microlab program with reference to method for Series 500 and Series 600. 5.10 reworded section. 12. Added additional references. 13.1.1.2. Changed expiration date for n-propanol working solution.</p>	<p>Modified by: PAK B1255      Date: 06/29/16</p>
<p>5.4.3 removed Microlab 500; 5.6. Removed Perkin Elmer from section and renumbered; 6.1.2 changed representative of manufacturer to a qualified vendor; Added 6.4 Balance Calibration Verification; 12-Removed all Perkin Elmer References; 13.1.1.1 changed 50mL stock solution; 13.2.1 changed loose cap issue to internal standard area count +/- 25% or mean</p>	<p>Modified by: MER B1466 on behalf of PAK B1255 Date: 2/9/17</p>
<p>5.3.5/5.4 removed serum and plasma information. 5.3.6 removed non biologicals. 5.9 i added sentence that “Aqueous reference materials must be from a different vendor or lot number than the calibrators.” Removed 7.2.2 serum/plasma and 7.2.3 non- biologicals</p>	<p>Modified by mer B1466 on 10/25/17 on behalf of PAK B1255</p>

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<p>5. added “All testing will be done in the controlled environment of the limited access toxicology lab.” Clarified that deficient samples are those with less than 2 mL. 5.3.1 added ‘The calibrators will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.’ 5.3.2 and 5.9.i added “At least one control will be purchased from an accredited reference material provider and meet the requirements of certified reference materials.” 5.3.5 deleted “Biological samples include blood.” Updated 6.3 to reflect new temperature monitoring system.</p>	<p>Modified by PAK 8/31/18</p>
<p>Section 1-Change “biological fluids or other liquid matrices” to “whole blood” Section 5.2-removed “or other container of liquid” Section 5.3.2-changed to “concentrations” Section 5.3.3-added “or ethanol free blood” Section 5.4 removed “or other liquid samples” Section 5.4.1 added “or L# (Lab number)” Section 5.4.2 removed “and placed with the corresponding labeled headspace vials” Section 5.4.3 removed “or other submitted container holding liquid”; added “or L#” after DR#</p>	<p>Modified by MER 5/6/19</p>
<p>Changed abbreviations Modified sample prep and Facesheet requirements</p>	<p>Modified by PAK 2/26/20</p>
<p>Section 4 was changed to reflect verification instead of validation. The calibration model was added as section 5.6.4. A requirement for verifying the whole blood blank be tested prior to use in casework was added.</p>	<p>Modified by PAK 03/30/21 /30/21</p>
<p>Clarified procedure on verifying whole blood used for blank.</p>	<p>Modified by PAK 4/29/22</p>
<p>Revised procedure for verifying whole blood blank. Modified requirements for reagent container labelling. Changed level of confidence to coverage probability. Added retention time requirements for identification of volatiles other than ethanol.</p>	<p>Modified by PAK 4/24/23</p>

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Clarified that serum and plasma will not be analyzed.	
Clarified that the area counts for the internal standard must be within 25% of the mean internal standard area counts for the calibrators for samples. Clarified post-maintenance instrument testing.	Modified by PAK 5/21/24