

# Alaska Scientific Crime Detection Laboratory

## Quantitative Alcohol Procedure Manual

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### SECTION 1: INTRODUCTION

The Alaska Administrative Code dictates aspects of the collection of blood and method of alcohol analysis. 13 AAC 063.110 gives information on the collection and handling of blood samples and 13 AAC 063.120 details methods of blood alcohol analysis that are appropriate for use in the State of Alaska.

The method used by this laboratory for quantitative ethanol analysis of body fluids and beverages is headspace gas chromatography with a flame ionization detector and mass spectrometer. A computer-interfaced instrument calibrates and analyzes specimens within a programmed sequence. The result is a comparison of unknown case samples with known concentrations of ethanol, with any variations in amount of injection corrected by the addition of an internal standard, n-propanol, in the diluent.

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### SECTION 2: EVIDENCE HANDLING

Evidence submitted to the Laboratory for ethanol analysis includes but is not limited to blood and suspected beverage alcohol samples. Blood is usually submitted in specific forensic alcohol collection kits containing gray top Vacutainer® tubes. Gray top tubes contain a sodium fluoride preservative and potassium oxalate anticoagulant. Gray top tubes do not require refrigeration because of their preservatives, but collection kits are refrigerated upon receipt to provide extra stability for any unpreserved samples and to comply with the Alaska Administrative Code. Forensic alcohol collection kits are also acceptable for submitting beverage samples. Suspected beverage alcohol samples are not required to be refrigerated.

Biological evidence is stored refrigerated at all times except during analysis. All evidence must be secured with access limited to the analyst when the analyst is not present.

When opening evidence for analysis, each layer of sealed packaging will be marked with the laboratory number, item number, date it was opened, and the initials of the analyst who opened it. The tube or item that is to be analyzed will be marked with the laboratory number, item number, date opened, and initials.

After opening the packaging, each blood sample will be checked for a subject name, which will in turn, be checked against the Request for Laboratory Services form for accuracy. Any discrepancies or lack of name will be documented in the analyst's notes.

The analyst's notes will also include: how the sample was packaged, if the packaging was sealed, the number and type of tubes submitted, any unusual aspects of the sample, and any broken seals or discrepancies. The date and time of collection and subject name will be recorded if that information is available from the blood tube or sealed container. The analyst will also record the date that the evidence is opened as the start of analysis date in the appropriate field of the notes function in the LIMS. The date of completion field will be left blank until the analysis is completed and is ready to forward the report on for technical review. The date of completion is entered when the analyst is ready to roll the draft complete milestone in the LIMS.

After opening, evidence items that were not submitted in a forensic alcohol collection kit will be secured in a laboratory provided package before returning. Any deviations from this practice will be documented in the notes. Samples that require drug toxicology testing will be packaged for shipment as described in the Washington Toxicology Send Out Working Instructions.

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Note: Blood and urine are biological materials and precautions associated with handling a biological hazard will be taken. Please refer to the Laboratory's Health and Safety Manual for more information regarding the handling of biological materials.

### Key Control

Refrigerator keys are located in their respective locks when not in use. Duplicate refrigerator keys are sealed in labeled envelopes and locked in the primary controlled substances cabinet in the reagent prep room # 2116. These keys can only be opened by the discipline supervisor or designee.

Each analyst also has a padlock with a unique key that can be used to secure evidence. The master key to these padlocks is in the possession of the Laboratory Manager.

Refer to the Laboratory Quality Assurance Manual for lab-wide Key Control Procedures.

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### **SECTION 3: EQUIPMENT AND INSTRUMENTATION**

#### Diluter/Dispenser

A Hamilton Microlab diluter/dispenser is used for sample dilution. The ETHANOL dilution method on the diluter/dispenser is set for a 100  $\mu\text{L}$  sample with 1000  $\mu\text{L}$  of internal standard diluent along with a 1000  $\mu\text{L}$  wash between samples. The B100 beverage dilution method on the diluter/dispenser is set for a 50  $\mu\text{L}$  sample with 4950  $\mu\text{L}$  of internal standard diluent along with a 5000  $\mu\text{L}$  wash between samples. The complete parameters for the methods can be found in the maintenance and calibration binders for each of the diluter/dispensers.

Each diluter/dispenser is calibrated annually by an external calibration provider that meets the requirements of accredited to ISO/IEC 17025:2005 with a scope of accreditation covering the calibration performed. Documentation of this maintenance and calibration information is kept in binders in the blood alcohol laboratory. Other maintenance and repair is performed, as needed, according to the manufacturer's recommendation and is documented in the maintenance log.

Internal performance checks are conducted on the diluter/dispensers between 5 and 6 months after the external calibration was performed. The Diluter Internal Check Worksheet that documents this internal performance check is completed, printed and stored in each diluter binder. The Diluter Internal Check Working Instructions detail the procedure to follow.

If an internal performance check does not pass, the diluter/dispenser will be removed from service until repair and external calibration is performed.

#### Headspace Gas Chromatograph/Mass Spectrometer

An Agilent gas chromatograph equipped with a headspace autosampler, a flame ionization detector and mass spectrometer is used for sample analysis. The instrument is interfaced with a computer and uses the Chemstation software to calibrate and analyze unknown case samples in the programmed sequence. A printed copy of the current instrument method used for analysis, along with any archived methods, is kept in the binder next to the instrument.

Repair and maintenance of the instrument and headspace autosampler is performed as needed in accordance with the manufacturer's recommendations. This is recorded in a binder kept in the instrument room.

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The following preventative maintenance tasks will be performed at the minimum frequencies outline below:

<u>Service</u>	<u>Minimum Frequency</u>
Oil Change	Every 6 months
Sample Probe	Every 2 years
Sample Loop	Every 2 years
Inlet Liner	Every 2 years
Source Cleaning	Every 2 years

Electronic copies of manufacturers' manuals for the diluter/dispensers and headspace gas chromatograph/mass spectrometers are located in the laboratory's internal network drive:

/discipline shares/chemistry/instrument manuals

### Validation Requirements

When new instrumentation is acquired, the manufacturer's representative will install and perform the initial set up of the instrument. The documentation will be stored with the discipline records in the LIMS.

Prior to use in case work a validation of the performance of the instrument will be performed. This documentation will be stored in the internal network drive:

/discipline shares/ blood alcohol/ blood alcohol validation

At a minimum, the validation of quantitative methods will evaluate precision, accuracy, limit of reporting, carryover, linearity, selectivity, and measurement uncertainty. When possible the validation will include a case comparison crossover study. The performance validation data will be stored in the discipline records for the life of the instrument. The performance data will also be forwarded to the Quality Assurance Manager.

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### SECTION 4: DILUENT, CALIBRATION STANDARDS AND CONTROLS

#### Internal Standard Diluent

Chemicals that meet American Chemical Society (A.C.S.) specifications will be used when available. Mix 0.2 mL of n-propanol in 2 liters of deionized water. Solution can be stored at room temperature and expires one year from date of preparation.

A newly prepared internal standard diluent is deemed suitable for use in casework when no ethanol is detected in the negative control and the mass spectrum identifies n-propanol when compared with a reference spectrum. The internal standard diluent's preparation is documented in the blood alcohol discipline's reagent log.

After a new lot of internal standard diluent is verified the following documentation is included in the blood alcohol discipline's reagent log:

- The chromatogram of the negative control showing n-propanol and no ethanol is detected
- The mass spectrum of n-propanol with a library match

#### Calibration Standards

NIST traceable aqueous ethanol standards at 0.020, 0.100, 0.200 and 0.500 g/100 mL are used for calibration. Calibration standards are purchased from an ISO/IEC 17025:2005 certified supplier. A critical supply vendor form is completed for each calibration standard.

Prior to using a new lot of calibration standard one vial from the lot will be run as a sample (in duplicate) to verify the lot falls within the manufacturer's stated concentration  $\pm$  the blood alcohol method's current expanded combined standard uncertainty at 95% coverage converted to g/100 mL units and rounded to three decimal places. The Ethanol Calibrator/Control Verification Worksheet will perform this calculation. A new shipment of the same lot does not require additional testing.

After a new lot of calibration standard is verified, the following documentation is included in the Calibration Standards binder:

- Certificate of Analysis
- Control Pack (See Section 7 for requirements)
- ETOH custom Chemstation Reports for the calibration standard being verified
- Ethanol Calibrator/Control Verification Worksheet

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### Negative Control (Blank)

The negative control is deionized water.

### Mixed Volatiles Control

Chemicals that meet American Chemical Society (A.C.S.) specifications will be used when available. To a 500 mL volumetric flask add 500  $\mu$ L of methanol, acetone, ethanol and isopropanol and 250  $\mu$ L acetaldehyde. Dilute to volume with deionized water. The solution can be stored at room temperature and expires one year from the date it was prepared.

A newly prepared mixed volatiles control is deemed suitable for use in casework when all chemicals in the mixture are detected with baseline separation during its first use and the mass spectra identify each component when compared with reference spectra. Each peak in the mixed volatiles control will be evaluated on a newly prepared standard.

After a new lot of mixed volatile control is verified the following documentation is included in the blood alcohol discipline's reagent log.

- The chromatogram of the mixed volatile control showing baseline separation of all components
- The mass spectrum of each component with a library match

### Whole Blood Ethanol Controls

Whole blood ethanol controls are purchased from a reputable supplier. A critical supply vendor form is completed for the whole blood controls.

Prior to using a new lot of whole blood control or a new shipment of a previously received lot, one vial of the received lot will be analyzed once (in duplicate). The averaged truncated result must fall within the manufacturer's expected range. If not, the lot will not be accepted for use. Then Ethanol Calibrator/Control Verification Worksheet will perform this calculation.

The following documentation for each lot is included in the Whole Blood Controls binder:

- Manufacturer control sheet
- Control Pack (See Section 7 for requirements)
- ETOH custom Chemstation Reports for the control being verified
- Ethanol Calibrator/Control Verification Worksheet

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### Aqueous Ethanol Controls

NIST traceable aqueous ethanol standards at 0.025 and 0.300 g/100 mL are used as low and high level ethanol controls. Aqueous ethanol controls are purchased from an ISO/IEC 17025:2005 certified supplier. A critical supply vendor form is completed for each ethanol control.

Prior to using a new lot of aqueous ethanol controls one vial from the lot will be run as a sample (in duplicate) to verify the lot falls within the manufacturer's stated concentration  $\pm$  the blood alcohol method's current expanded combined standard uncertainty at 95% coverage converted to g/100 mL units and rounded to three decimal places. The Ethanol Calibrator/Control Verification Worksheet will perform this calculation. A new shipment of the same lot does not require additional testing.

After a new lot of aqueous ethanol controls is verified, the following documentation is included in the Aqueous Ethanol Control binder:

- Certificate of Analysis
- Control Pack (See Section 7 for requirements)
- ETOH custom Chemstation Reports for the control being verified
- Ethanol Calibrator/Control Verification Worksheet

### Beverage Controls

Purchase a non-carbonated wine and dispense aliquots into approximately 2mL vials. Label the vials with the beverage control lot and date. The lot number is incremental. If the previous beverage control lot was lot 1, the next will be lot 2. Create a lot number sheet listing the name and type of wine, the percent alcohol from the bottle's label, the transfer date and analyst that created the lot along with the lot number.

The beverage control is analyzed as a sample (i.e. in duplicate) in two beverage runs. The final results of these analyses are averaged and this average is used as the starting lot concentration in the beverage control worksheet. The alcohol by volume detected should be between 5-20%.

The final results from the two beverage runs must fall within a predefined percentage of their average. This percentage is the beverage alcohol method's current expanded combined standard uncertainty at 95% coverage converted to %v/v and rounded to one decimal place.

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After a new lot of beverage control is created, the following documentation is included in the Whole Blood Controls binder:

- Beverage Lot Number Sheet
- Control Pack (See Section 7 for requirements)
- ETOH custom Chemstation Reports for the control being verified (from two beverage runs)
- Ethanol Control Worksheet containing values of the control being verified

### Date Documentation of Controls and Calibration Standards

When a new shipment of controls or calibration standards is received by the laboratory, the date received is marked on the certificate of analysis. After the new shipment is verified, the date in which they are put in use is marked on the certificate as well. Finally, when the last control or calibration standard of a shipment is consumed, the end of use date is marked on the certificate of analysis.

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### SECTION 5: ANALYSIS PROCEDURE

#### Sample Selection

Refer to Policy 11 (Selection of Items for Analysis) in the laboratory Quality Assurance Manual for a summary of item selection practices used for this discipline.

#### Sampling Plans

In the alcohol testing section it is common to receive items that contain multiple containers (units) submitted as a single item. In these instances, the analyst must determine which and how many of these units must be sampled and analyzed. The sampling plan listed below describes how the alcohol testing section makes sampling decisions.

Blood samples are usually collected in forensic alcohol testing kits containing one to four gray top tubes. Blood collected from an individual sequentially into the same type of tube can be treated as one item even when only one tube is sampled. The analyst report will reflect the number of tubes contained in the item along with the result of that item.

Blood collected into different types of tubes will be considered as separate items. Any additional tubes in the item will be indicated in the notes field of the LIMS.

Beverage samples can often have multiple units collected and submitted at the same item. The analyst's report will indicate what was present in each item and what was tested. For example, two 10 mL gray top tubes containing a yellow cloudy liquid were submitted. The analyst would select one tube for analysis and their report would read 2 10 mL gray top tubes of yellow, cloudy liquid one tube analyzed. The ethanol result would then be reported for the tube that was analyzed.

In instances where more than one of the units must be sampled to meet critical volumes listed in AS 04.16.200 the analyst must perform full testing on all units required or use a statistical sampling plan. The statistical sampling plan used by the alcohol testing section is the hypergeometric method. The confidence level associated with this sampling plan must be 95% confidence that at least 90% of the units contain the analyte. Appendix B is the sampling plan employed. The decision about whether full testing is required or a sampling plan is employed will be made on a case by case basis. When a sampling plan is employed details will be provided in the notes of the case record and referenced on the report.

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### Sample Preparation

Allow calibration standards, controls, and case samples to warm to room temperature prior to sampling. Prepare two vials for each sample and control that is to be analyzed. Set up one vial each for the blanks, mixed volatiles control and calibration standards. A blank is run as the first sample in each sequence followed by the calibration standards. A blank and the mixed volatiles control is run following the calibration standards. A 0.300 aqueous ethanol control, whole blood or ethanol control and a 0.025 aqueous ethanol control are run in duplicate immediately prior to and following a batch of case samples. The maximum number of case samples per run is 15. A sample blood alcohol sequence can be found in the instrument binder located in the instrument room.

Label the autosampler vials, using indelible marker, with the appropriate lab number, calibration level, or control.

Begin sample preparation by priming the diluter/dispenser with the internal standard diluent. Ensure there is sufficient internal standard diluent in the bottle to dilute the entire run. Make sure that there are no bubbles in either syringe. Select the ETHANOL program and follow the instructions as it directs you through the setup. Ensure that each sample is mixed by inverting gently several times prior to sampling. Dispense 1000  $\mu\text{L}$  of internal standard diluent, along with 100  $\mu\text{L}$  of sample, calibration standard, or control into the headspace vials using the diluter/dispenser. Wipe the tip of the dispenser between each sample. Cover the vials with the caps and crimp tightly onto the vials. Continue this process for all samples in the run. If the quality of a sample won't allow for dilution the analyst will notify the submitting agency that analysis was not performed.

Suspected beverage alcohol samples are run in separate batches than whole blood and serum samples. In a beverage alcohol run, the whole blood controls that are run before and after case samples are replaced with the beverage control. One vial of the beverage control is diluted 1:100, twice to make two primary dilutions. These dilutions are tested in duplicate, once at the beginning of the run and once at the end. The lot number of the beverage control will be listed on the Ethanol Control Worksheet that will be scanned with each case file into the LIMS.

Beverage samples may need a preliminary dilution before the diluter/dispenser process. These samples may be diluted 1:100 with deionized water. The dilution factor can then be adjusted by the analyst if needed based on the test results. Unless noted otherwise in the LIMS the dilution is 1:100.

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### Instrument Preparation

Sequences are imported to the Chemstation software using the LIMS. Instructions on sequence importation and the method parameters are located in the binder next to the instrument. For additional instructions on how to use the Chemstation software, refer to the manufacturer's instructions. Place all vials into the auto sampler tray in the order they are listed in the sequence table.

Each day, prior to running a sequence, the mass spectrometer is calibrated using the Autotune program. A successful calibration is indicated when the instrument is able to assign the proper masses to the Perfluorotributylamine (PFTBA) calibration standard. The tune evaluation must pass in order to use the instrument.

A copy of the Autotune report and tune evaluation is included in the control packet associated with each case in the run.

### Instrument Analysis

The ethanol retention time is determined by the method. The sequence is designed to set the retention time of ethanol to that of the first ethanol calibrator. Subsequent peaks must fall within plus or minus 5% of that retention time per the method. Any peaks not falling in this range are not considered to be ethanol and will not be quantitated as such.

The mass spectrum generated for the ethanol peak is included on the ETOH custom Chemstation report. The analyst will ensure that the mass spectrum of ethanol contains the ions 31, 45 and 46.

Additional peaks (if investigated by the analyst) will be documented in the LIMS with the mass spectrum of the peak and any library matches. This will be done for both duplicates analyzed.

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### **SECTION 6: PASSING CRITERIA – CASEWORK CONTROLS**

#### Acceptability Requirements

The aqueous controls, whole blood controls, beverage controls and case samples are run in duplicate and calculated in the same manner. Duplicate results are truncated to 4 decimal places and averaged. The difference between the truncated 4 decimal place numbers is divided by the average to determine the RPD. The RPD must be 3% or less for the average to be used.

For aqueous and whole blood controls the average truncated to 3 decimal places is compared to the expected value. The passing range is equal to the expected value  $\pm$  the blood alcohol method's current expanded combined standard uncertainty at 95% coverage converted to g/100 mL units and rounded to three decimal places.

For beverage controls the average truncated to 3 decimal places is converted to alcohol by volume, as outlined in Section 8 Reporting, and is compared to the expected value. The passing range is equal to the expected value  $\pm$  the beverage alcohol method's current expanded combined standard uncertainty at 95% coverage converted to %v/v units and rounded to one decimal place.

For aqueous controls, the expected value is the listed concentration. For whole blood and beverage controls, the expected value is the current running average of the lot.

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### SECTION 7: CASE FILES

Case files are stored electronically in the LIMS system. Each case file is composed of the control pack which includes:

- The Autotune report and Tune Evaluation
- Calibration curve
- The ETOH custom Chemstation report for the blanks, mixed volatiles control, each calibration standard, and each control analyzed
- A copy of the sequence table for the run
- Ethanol Control Worksheet

The case file also includes the following in addition to the control pack listed above:

- The ETOH custom Chemstation report for each case sample duplicate
- The analyst's worksheet
- The Laboratory's Request for Laboratory Services
- Chain of Custody Documentation
- The Crime Laboratory report
- Any case related correspondence

#### Ethanol Control Worksheet

The Ethanol Control Worksheet is a separately controlled document which contains current calibrator and control lot information, control passing ranges, and identification and current use of diluter/dispensers. It is programmed to calculate whether control data from a particular analysis sequence meets the acceptance criteria outlined in Section 6. The Ethanol Control Worksheet is also used to track all control data for future uncertainty evaluations.

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### SECTION 8: REPORTING

A LIMS system is used for compiling all case and run data into each case file and generating crime laboratory reports. There are three types of requests in the LIMS for forensic alcohol testing: blood alcohol, beverage alcohol and serum/plasma alcohol. The analyst must edit the request in the LIMS to reflect the sample they are testing. Instructions for importing data are located in a binder next to the instrument. After the data is imported the analyst will review the imported data and case file. Upon completion of the run verify that:

- Blanks demonstrate no measurable ethanol.
- The mixed volatiles control has baseline separation of all components and that the mass spectrum of ethanol contains the ions 31, 45, and 46.
- Calibration standards results (after reprocessing) are within  $\pm 0.005$  or 5%, whichever is greater, of their expected value.
- All controls passed (See Section 6)

If a blank, mixed volatiles control, calibration standard, aqueous ethanol control, beverage control or whole blood control is out of range or does not meet the above requirements the case samples will be re-diluted and reanalyzed.

If duplicate analyzed values of a case sample do not agree within  $\pm 3\%$  and the mean of the duplicates is greater than the limit of reporting, then the sample will be diluted a second time and analyzed. If the second dilution's analyzed values do not fall within  $\pm 3\%$ , the sample may be diluted a third time.

Should the analyzed values of the third dilution not fall within  $\pm 3\%$ , the sample will be reported as: "**Sample quality insufficient for quantitation.**" This report wording will also be used in situations where sample quality prohibits preparation due to dilutor clogging.

For blood alcohol analyses, the concentration will be reported as follows:

- Alcohol values less than or equal to 0.009 g/100mL: "**None Detected**"
- Alcohol values greater than or equal to 0.010 and less than 0.020 g/100mL: "**Less than 0.020 g/100mL**"

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- Alcohol values greater than or equal to 0.020 g/100 mL and less than or equal to 0.500 g/100 mL: Average of the two analyzed values truncated to three decimal places.
- Samples with alcohol values over 0.500 g/100mL will be diluted and rerun.

For beverage alcohol analyses, the mean of the duplicates is multiplied by the dilution used and then converted to volume of ethanol per volume of liquid by dividing by the density of ethanol. For example:

$$0.050 \text{ g/100 mL (ETOH concentration)} \times 100 \text{ (dilution factor)} \div 0.789 \text{ g/mL (density ETOH)} \\ = 6.3 \text{ mL/100 mL} = 6.3\% \text{ v/v}$$

If the mean of the duplicates is below the limit of quantitation (0.020 g/100 mL) and the beverage sample was diluted, the sample must be reanalyzed with a lower dilution factor or none at all.

The beverage concentration will be reported as follows:

- If greater than or equal to 0.025% v/v: report ethanol concentration.
- If less than 0.025% v/v but greater than or equal to 0.012% v/v: **“Positive ethanol, less than 0.025% v/v”**
- If less than 0.012% v/v: **“None Detected”**
- The number of decimal places used when reporting beverage alcohol concentrations will be based on the pre-dilution factor as follows:
  - 100 times pre-dilution = 1 decimal place
  - 10 times pre-dilution = 2 decimal places
  - No pre-dilution = 3 decimal places

The expanded combined standard uncertainty at 95% coverage is included with any reported concentrations. This is reported in the same units as the ethanol result. For additional information regarding measurement of uncertainty see Appendix A.

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The analyst will then affix their electronic signature. After reports are signed by the analyst they are forwarded to a qualified analyst for technical and administrative review. The evidence must be returned to the evidence vault or another laboratory member prior to administrative review. At the completion of analysis, the glass headspace vials will be disposed of in the appropriate biohazard container and the evidence returned to the evidence section if no other testing is required.

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### **SECTION 9: PROFICIENCY TESTING**

The guideline for satisfactory completion is based on the manufacturer's expected results and the average of all ASCLD/LAB respondents. This means  $\pm 10\%$  or  $\pm 2$  standard deviations of the average of all ASCLD/LAB respondents for samples. The current reported uncertainty of measurement range can be considered when evaluating the proficiency test results. Proficiency test results are maintained by the Quality Assurance Manager.

Blood alcohol results provided to the proficiency test provider will be reported in the same manner as calculated for casework.

Proficiency test samples are transferred to the evidence vault prior to administrative review and the quality manager will destroy the samples after the results have been received from the proficiency test provider.

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### **SECTION 10: PROVIDING OPINIONS FOR COURT PROCEEDINGS**

In general, calculations based on a blood alcohol concentration are elicited as expert opinion during testimony in court proceedings. The Interpretation of Alcohol Results Manual outlines the interpretation of the results of blood and breath alcohol measurements. In some instances, a written opinion is requested prior to court proceedings. In this case, the analyst will prepare a memo containing their opinion and issue it to the requesting agency. For cases in which a blood alcohol analysis was performed within the laboratory, the prepared memo will be scanned into the imaging module for the relevant case. Memos not relevant to a specific analysis performed within the laboratory will be scanned into the imaging module for the case B-OPINIONYYYY (where YYYY is the year that the memo was written) located within the breath alcohol database. A report will not be generated for expert opinion.

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### SECTION 11: TOXICOLOGY SEND OUTS

Toxicology services provided by Washington State Patrol to the Alaska Scientific Crime Detection Laboratory are funded through a National Highway Traffic Safety Administration (NHTSA) grant. The Alaska Scientific Crime Detection Laboratory does not currently send evidence to Washington in non-driving offenses for drug toxicology analysis. Evidence will be sent to Washington State Patrol for drug toxicology analysis if the case meets one of the following criteria:

- A Drug Recognition Expert (DRE) evaluation was performed
- The submitting agency requests a drug toxicology analysis and the case involves a driving related accident leading to the serious injury or fatality of a person other than the driver
- The submitting agency requests a drug toxicology analysis, the case involves a driving related offense, and a blood alcohol request is not made
- The submitting agency requests a drug toxicology analysis, the case involves a driving related offense, a blood alcohol request is made and its result is less than 0.100 g/100 mL

Note: At the blood alcohol discipline supervisor's discretion, evidence from traffic related cases not meeting the criteria outlined above may also be sent to Washington State Patrol for drug toxicology analysis when the current NHTSA grant allows.

Before staff members prepare evidence for drug toxicology send outs, they will ensure that any blood alcohol requests for the associated cases are complete. Instructions for performing Washington toxicology send outs and entering Washington toxicology analysis reports in the LIMS are found in the Toxicology Send Outs Working Instructions.

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### SECTION 12: ABBREVIATIONS

AEC:	Aqueous Ethanol Control
FID:	Flame Ionization Detector
GC:	Gas Chromatograph
HSS:	Headspace sampler
PFTBA:	Perfluorotributylamine
MS:	Mass Spectrometer
MV:	Mixed volatiles
RLS:	Request for laboratory services form
RPD:	Relative percent difference
WBC:	Whole blood control

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# Alaska Scientific Crime Detection Laboratory

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## Quantitative Alcohol Procedure Manual

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### APPENDIX A: UNCERTAINTY OF MEASUREMENT

The estimation of the uncertainty of measurement of the ethanol concentration in samples has been performed. The initial study performed in 2012-2013 has been documented in a report titled "Forensic Alcohol Estimate of the Uncertainty of Measurement December 20, 2013 Blood Ethanol and Beverage Ethanol". This report is stored in the internal network drive of the Forensic Alcohol discipline share folder and a copy is kept in the Quality Assurance Records.

The reports address the following elements:

- Statement of the measurand
- Statement of the measurement traceability
- Equipment used
- All uncertainty components considered
- All significant uncertainty components and their evaluation
- Data used to estimate repeatability and/or reproducibility
- All calculations
- The combined standard uncertainty, the coverage factor, the coverage probability, and the resulting expanded uncertainty
- The schedule to review and/or recalculate the measurement of uncertainty.

**The current expanded combined standard uncertainties at 95% coverage for blood and beverage ethanol analysis are  $\pm 4.2\%$  and  $\pm 4.5\%$  for beverage ethanol analysis at the Alaska Scientific Crime Detection Laboratory.**

An ongoing estimation of the uncertainty of measurement is performed utilizing data from all case batches. This information is stored in the Ethanol Control Worksheet stored in the internal network drive of the Forensic Alcohol discipline share folder.

The data from this ongoing estimation of the uncertainty of measurement will be reviewed and an updated estimation of the uncertainty of measurement reports will be written at least 36-39 months from the previous report or if any significant change in the expanded uncertainty is detected.

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### APPENDIX B: Hypergeometric Sampling Plan\*

The use of the sampling plan requires that all units appear to be homogenous. If any results are different than the rest, the analyst must re-evaluate the population. The report will reference the sampling plan utilized and the confidence levels and corresponding inferences of the population. The report will state what was received and clearly state the results/conclusions.

<u>Population Size</u>	<u>Sample Size</u>
1-12	All
13-20	12
21-30	15
31-40	18
41-50	19
51-60	20
61-70	21
71-80	22
81-100	23
101-130	24
131-180	25
181-270	26
271-470	27
471-1000	28

Based on statistical probability, there is 95% confidence that at least 90% of the units contain ethanol.

\*Information is taken from enfsi-dwg sampling calculator 2012 referenced in the ASCLD/LAB Policy on Sampling, Sampling Plans and Sample Selection.

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### APPENDIX C: REVISION HISTORY

2016 R3 page	2016 R2 Page	Location	Revision made
3	3	Section 2: Evidence Handling, first sentence of last paragraph	Removed sentence because proper sealing of evidence is already addressed in Quality Assurance Manual. Added two new sentences referring to laboratory provided packages
3	3	Section 2: Evidence Handling, second sentence of last paragraph	Added "drug" in front of "toxicology testing" and included reference to Washington Toxicology Send Out Working Instructions
7-9	7-9	Section 4: Diluent, Calibration Standards and Controls, Calibration Standards, Whole Blood Ethanol Controls, and Aqueous Ethanol Controls sub-sections	Made references to the new Ethanol Calibrator/Control Verification Worksheet
17	17	Section 8: Reporting	Added sentence beginning with "If the mean of", deleted "(limit of quantitation)" and "(limit of reporting)" from beverage reporting bullets