

# Alaska Scientific Crime Detection Laboratory

## Ethanol Quantitation Procedure Manual

Effective: 6/3/2024

Version: 8.0

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### Introduction

The Alaska Administrative Code dictates procedures for 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.

**Note:** Forensic Alcohol staff do not receive training in or conduct blood collection.

The method used by this laboratory for identification of volatile substances and quantitative ethanol analysis of body fluids and beverages is headspace gas chromatography with a flame ionization detector and mass spectrometer.

### Abbreviations and Definitions

<b>AAC:</b>	Alaska Administrative Code; a set of Alaska-specific legal regulations
<b>ABV/abv:</b>	Alcohol by volume, a unit of concentration commonly used for alcoholic beverages
<b>ACS:</b>	American Chemical Society
<b>AEC:</b>	Aqueous ethanol control
<b>ANAB:</b>	ANSI-ASQ National Accreditation Board
<b>AS:</b>	Alaska Statutes
<b>EtOH:</b>	Ethanol
<b>FA:</b>	Forensic Alcohol
<b>FID:</b>	Flame ionization detector
<b>g:</b>	Grams, a unit of mass
<b>GC:</b>	Gas chromatography
<b>HSS:</b>	Headspace sampler
<b>ISO/IEC:</b>	International Organization for Standardization/International Electrotechnical Commission; an independent organization that develops international standards
<b>% v/v:</b>	The volume of pure ethanol per volume of the entire liquid (expressed as a percentage), a unit of concentration commonly used for alcoholic beverages
<b>mL:</b>	Milliliter, a unit of volume
<b>MS:</b>	Mass spectrometry
<b>MSD:</b>	Mass selective detector, the detector used for mass spectrometry
<b>MV/MVC:</b>	Mixed volatiles control, a type of liquid control used in blood alcohol analysis
<b>m/z:</b>	Mass-to-charge ratio, a value used in mass spectrometry

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<b>NHTSA:</b>	National Highway Traffic Safety Administration
<b>NIST:</b>	National Institute of Standards and Technology
<b>PFTBA:</b>	Perfluorotributylamine, a mass calibration standard for a mass selective detector
<b>PPE:</b>	Personal protective equipment (e.g., nitrile gloves, face shield)
<b>Ret Time:</b>	Peak retention time determined via gas chromatography, expressed in minutes
<b>RLS:</b>	Request for Laboratory Services form
<b>RPD:</b>	Relative percent difference, a measure of repeatability
<b>SCDL:</b>	Alaska Scientific Crime Detection Laboratory
<b>µL:</b>	Microliter, a unit of volume
<b>Version:</b>	Controlled document revision number after SharePoint controlled document migration (October 2021)
<b>WBC:</b>	Whole blood control

## Equipment and Instrumentation

### Method Limitations

The test method is designed to detect liquid ethanol concentrations of 0.010 g/100 mL and above, as well as quantitate concentrations between 0.020 g/100 mL and 0.500 g/100 mL. For a beverage alcohol solution using a pre-dilution factor of 100, this is equivalent to a beverage between approximately 2.5% v/v and 63.4% v/v.

The matrices approved for use with this method are human blood, serum, and plasma, as well as suspected alcoholic beverages. Urine samples are not accepted by the laboratory.

### Diluter/Dispenser

A Hamilton Microlab diluter/dispenser is used for sample dilution. The table below lists the dilution methods available.

Method Name	Purpose	Diluent
ETHANOL	Primary dilution method	Internal Standard Diluent
B100	100 times pre-dilution	Deionized water
B10	10 times pre-dilution	Deionized water

Method details are listed in the Microlab 500 Dilution Methods and Microlab 600 Dilution Methods appendixes of this manual.

### Diluter Calibration

Each in service diluter/dispenser is calibrated by an external calibration provider with a current ISO/IEC 17025 accreditation, and a scope of accreditation covering the calibration performed.

External diluter calibrations, calibration certificates, and applied stickers must meet the following requirements:

- As Found (Initial) and As Left (Final) testing
- Where adjustment was performed, a clear indication that it was performed
- Calibration over the intended measurement range (may be by volume or percentage of stroke, the travel distance of the piston), at minimum:
  - o 3 different volumes/percent strokes
  - o Desirable values are 10%, 50%, and 100% of the stroke (corresponding volumes depend on the size of the installed burette)
    - If % of stroke is used, the corresponding volumes must still be listed on the calibration certificate
  - o 3 or more measurements at each level

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- Calibration uncertainty is listed on the calibration certificate
- The period of validity is one year after calibration

External calibration certificates are located in the laboratory's SharePoint document library.

### Diluter Maintenance

Other maintenance and repairs are performed, as needed, and are documented in the maintenance log stored on the laboratory's SharePoint document library. If a diluter is suspected of having a performance issue, the diluter will be placed marked as out of service until such a time as repair can be completed. The primary method of evaluating a diluter for return to service is by using the diluter to prepare calibrators and controls for ethanol quantitation; however, other forms of evaluation may be appropriate depending on the service performed.

Electronic copies of manufacturers' manuals for the diluter/dispensers are located on the laboratory's SharePoint document library.

### Headspace Gas Chromatograph/Flame Ionization Detector/Mass Spectrometer (HSS-GC-FID-MS)

An Agilent gas chromatograph equipped with a headspace autosampler, a flame ionization detector, and mass spectrometer are used for sample analysis. The instrument is interfaced with a computer and uses 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 on the laboratory's SharePoint document library.

### HSS-GC-FID-MS Maintenance

Repair and maintenance of the instrument and headspace autosampler is performed as needed in accordance with the manufacturer's recommendations. This is recorded in the instrument maintenance log on the laboratory's SharePoint document library.

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

<u>Service</u>	<u>Minimum Frequency</u>
Scroll pump tip seal replacement	Annually
Sample Probe	Every 2 years
Sample Loop	Every 2 years
Inlet Liner	Every 2 years
Replace FID Collector Assembly	Every 4 years
Replace FID Jet	Every 4 years

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Electronic copies of manufacturers' manuals for the headspace instruments are in the laboratory's SharePoint document library.

If a performance issue is suspected, the instrument will be clearly labeled as Out of Service until the issue is resolved. The primary method of performance evaluation for this instrument type is running a full ethanol quantitation sequence; however, more limited testing may be appropriate to return the instrument to service depending on the issue or maintenance performed

### Integration

Integration is primarily a concern on the flame ionization detector (FID) signal as this signal is used for quantitation. The goals of integration for this method are as follows:

1. Baseline separation of all components of the mixed volatiles standard.
2. Integration is such as that the area of the ethanol and/or internal standard peaks are captured appropriately and compensates for rising or falling baseline.
3. Integration is consistent for replicates of the same solutions/samples.

Default integration parameters are set to generally achieve these goals without the need for editing.

#### *Default Integration Parameters*

Instrument software will integrate peaks based on the integration parameters set in the instrument software. The default integration parameters used are listed in the table below.

Integrator Event Name	Value	Time
Initial Area Reject	0	Initial
Initial Peak Width	0.030	Initial
Shoulder Detection	OFF	Initial
Initial Threshold	13.0	Initial

#### *Changing Integration Parameters*

If the default integration has not achieved one or more of the goals of integration listed above, the sequence method (the version of the method associated with the specific analysis sequence) may have the integration parameters adjusted until the integration meets the goals listed above. This is performed in the offline (data processing) software.

**IMPORTANT:** Changes to integration are preferentially applied to the entire sequence. Integration changes to a single injection should only be considered if changes to the entire sequence have been investigated and found to be insufficient. Changing the integration parameters requires regeneration of the calibration table followed by reprocessing. Consult the Technical Lead if assistance is needed.

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The most adjusted integration parameters are:

1. Initial Threshold (Slope sensitivity)
2. Initial Peak Width
3. Initial Area Reject

If it is not possible to achieve the integration goals listed above with reasonable effort, contact the Technical Lead.

### *Recalibration and Reprocessing After Changing Integration Parameters*

Once the integration achieves the goals listed above, save the sequence method to retain the integration parameters for that sequence, reprocess the sequence to update the calibration table, then reprocess again with all injections as Samples.

Modified integration parameters must be clearly specified for each impacted sample. If the integration parameters for the entire run were modified, this information may be noted in the control pack. If only case sample integration was modified, the changed parameters must be recorded in the notes for each case for which they are used.

### *Mass Spectral Libraries*

The discipline's in-house library and/or outside libraries from reputable sources may be used during mass spectral searches. The library search report will list which library was used for each sample analyzed. Libraries will be located on each instrument computer in the following directory:  
C:\DATABASE\.

Mass spectra obtained from working standards will be added to the in-house library after verification. Background subtraction will be used before adding a spectrum to the library if column bleed or other interfering ions are present. Newly added library entries will be uniquely identified by listing the name of the standard (e.g., the specific chemical name) and the manufacturer lot number in the title of the entry.

The discipline's in-house library will be named SCDL-FA-YYMMDD where YYMMDD is the date in which that library version was put into use. Whenever a new entry is added to the discipline's in-house library, the updated version will be copied to each instrument computer and will replace the previous version.

The contents of outside libraries will not be edited in any way; however, if a newer version of an outside library is obtained, it will replace the previous version on every instrument computer. For tracking purposes, the names of outside libraries will include –YYMMDD where YYMMDD is the date in which that library version was put into use.

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Backups of current and previous libraries (both outside and in-house) will be placed on the laboratory's SharePoint document library.

### Validation Requirements

When new instrumentation is acquired, the manufacturer's representative will install and perform the initial set up of the instrument.

Prior to use in casework a validation of the performance of the instrument will be performed. A validation plan must be approved by the discipline supervisor or their designee before the validation study commences. 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.

Validation reports are located on the laboratory's SharePoint document library.

## Reagents, Standards, and Controls

### Reagents

#### Negative Control

Ingredients:	The negative control is deionized water.
Storage:	Store at room temperature and there is no expiration.
Verification:	None
Documentation:	None

#### Internal Standard Diluent

Ingredients:	Mix 0.2 mL of n-propanol in 2 liters of deionized water. (Chemicals that meet American Chemical Society (ACS) specifications will be used.)
Storage:	Store at room temperature and expire one year from date of preparation.
Verification:	A new lot of internal standard diluent is verified by injecting one negative control sample prepared with the new diluent. This sample can be analyzed without any accompanying controls or calibrators. The newly prepared lot 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.
Documentation:	The following verification documentation is kept in the reagent logbook: <ul style="list-style-type: none"><li>• The chromatogram of the negative control showing n-propanol and no ethanol detected</li><li>• The mass spectrum of n-propanol with a library match</li><li>• The date of first and last use for each lot of internal standard diluent</li></ul>

## Qualitative Standards

### Mixed Volatiles Standard

- Supplier:** A volatiles standard mix containing methanol, ethanol, isopropanol, and acetone is purchased from a supplier with a current ISO/IEC 17025 and ISO/IEC 17034 accreditation.
- Storage:** Mixed Volatile Standards are stored according to the manufacturer's recommendation when not in use and can be used until the manufacturer's expiration date. After opening an ampule, the contents will be transferred to a sealed vial for storage. The contents of opened ampules will be discarded after three months.
- Verification:** A new lot of mixed volatiles standard is verified by injecting one sample of the standard. This sample can be analyzed without any accompanying controls or calibrators. The standard is deemed suitable for use in casework when all chemicals in the mixture are detected with baseline separation and the mass spectra identify each component when compared with reference spectra.
- Documentation:** The following documentation is included in the Standards and Controls binder:
- Certificate of Analysis with the received date, date of first use, and date of last use for the lot.
  - The chromatogram of the mixed volatile standard showing baseline separation of all components
  - The mass spectrum of each component with a library match

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### Volatile Primary Standards

- Supplier:** Primary standards are purchased from a supplier with a current ISO/IEC 17025 and ISO/IEC 17034 accreditation.
- Storage:** Primary standards are stored according to the manufacturer's recommendation when not in use and can be used until the manufacturer's expiration date.
- Verification:** A new lot of primary standard is verified by injecting one sample of the new standard. This sample can be analyzed without any accompanying controls or calibrators. The standard is deemed suitable for use in casework when the mass spectra identify each component when compared with reference spectra. If the standard is not in the in-house mass spectral library, it will be added after verification.
- Documentation:** When a primary standard is used in casework, the lot number will be recorded in the case file. The following documentation is included in the Standards and Controls binder:
- Certificate of Analysis with the received date, date of first use, and date of last use for the lot.
  - The chromatogram of the standard
  - The mass spectrum of each component with a library match

## Quantitative Standards and Controls

### Calibration Standards

- Supplier:** 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 a supplier with a current ISO/IEC 17025 and ISO/IEC 17034 accreditation.
- Storage:** Calibration standards are stored according to the manufacturer's recommendation when not in use and can be used until the manufacturer's expiration date. Calibration standards must be used within one day after opening. Any remaining contents of the standard ampule will be discarded.
- Verification:** 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  $\pm 0.005$  g/100 mL or 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, whichever is greater. The Ethanol Calibrator/Control Verification Worksheet will perform this calculation.
- Documentation:** The following documentation is included in the Standards and Controls binder:
- Certificate of Analysis with the received date, date of first use, and date of last use for the lot.
  - ETOH custom Reports for the calibration standard being verified
  - Ethanol Calibrator/Control Verification Worksheet

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## Aqueous Ethanol Controls

- Supplier:** 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 a supplier with a current ISO/IEC 17025 and ISO/IEC 17034 accreditation. Aqueous ethanol controls are purchased from a different supplier than calibration standards.
- Storage:** Aqueous ethanol controls are stored according to the manufacturer's recommendation when not in use and can be used until the manufacturer's expiration date. Aqueous ethanol controls must be used within a day after opening. Any remaining contents of the standard ampule will be discarded.
- Verification:** 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  $\pm 0.005$  g/100 mL or 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, whichever is greater. The Ethanol Calibrator/Control Verification Worksheet will perform this calculation.
- Documentation:** The following documentation is included in the Standards and Controls binder:
- Certificate of Analysis with the received date, date of first use, and date of last use for the lot.
  - ETOH custom Reports for the control being verified
  - Ethanol Calibrator/Control Verification Worksheet

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### Whole Blood Ethanol Controls

- Supplier:** Whole blood ethanol controls are purchased from a reputable supplier.
- Storage:** Whole blood controls are stored according to the manufacturer's recommendation when not in use and can be used until the manufacturer expiration date.
- Verification:** Prior to using a new lot of whole blood control, one vial of the received lot will be analyzed once (in duplicate). The averaged, truncated result must fall within +/- 15% of the manufacturer's target value. If not, the lot will not be accepted for use. The Ethanol Calibrator/Control Verification Worksheet will perform this calculation.
- Documentation:** The following documentation is included in the blood and beverage controls binder:
- Manufacturer control sheet
  - ETOH custom Reports for the control being verified
  - Ethanol Calibrator/Control Verification Worksheet

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## Beverage Control

- Supplier:** Laboratory prepared from non-carbonated wine. See preparation instructions below.
- Storage:** Beverage controls are refrigerated when not in use. There is no expiration date for a lot of beverage control.
- Verification:** The beverage control is analyzed as a sample (i.e., in duplicate) in two beverage runs. The 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 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.
- Documentation:** The following documentation is then included in the Blood and Beverage Controls binder until it is no longer in use and digitally archived:
- Beverage Lot Number Sheet with the date of first use and date of last use for the lot.
  - ETOH custom Reports for the control being verified (from two beverage runs)
  - Ethanol Control Worksheet containing values of the control being verified

### *Beverage Alcohol Control Preparation Instructions*

Purchase a non-carbonated wine and dispense aliquots into approximately 2 mL vials. Label the vials with the beverage control lot and date. The lot number is incremental. For example, 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, analyst that created the lot, the lot number, and the control pack identifiers for the two confirmation beverage runs (see below).

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### *Beverage Training and Control Preparation Materials*

Manufactured beverage samples are kept by the toxicology discipline for training materials and for use in the preparation of the beverage control. These samples are tracked and stored with the Seized Drug Training Materials. See the Seized Drug Procedure Manual for additional information on tracking and storage procedures.

### **Digitally Archiving Verification Records**

When a calibration standard, primary standard, mixed volatile standard, internal standard diluent lot, aqueous ethanol control, whole blood control, or beverage control is no longer in use, the hardcopy of its verification record will be scanned and placed in the laboratory's SharePoint document library. After ensuring that the scanned record is legible and complete, the hardcopy will be destroyed. Records will be digitally archived annually, at minimum.

### **Storage and Use of Purchased Chemicals**

#### Definition and Quality Requirements

Purchased chemicals are used for reagent preparation and cleaning. Examples of purchased chemicals are solvents, acids and bases, and dry/solid chemicals. Purchased chemicals used for casework including reagent preparation will be ACS grade or better.

#### Storage Conditions and Expiration Dates

Chemical manufacturers specify the storage conditions of chemicals. Chemicals should be stored according to the manufacturer's storage conditions, including consideration of any chemical hazards (e.g., no storage of oxidizers with flammables, organics, etc.). Refer to the Health and Safety Manual for more information.

Common oxidizers: Nitrites (e.g., Nitric acid), Peroxides (e.g., Hydrogen peroxide), Perchlorates (e.g., Perchloric acid), Permanganates (e.g., Potassium permanganate)

Chemical manufacturers may print expiration or retest dates on chemical containers. A chemical should not be used for casework beyond the manufacturer expiration date. Additionally, reagents may not have an expiration date later than that of the earliest component expiration date.

Example: If a reagent is prepared that typically has a 1-year expiration using a solvent that expires in 8 months, the prepared reagent must also expire in 8 months.

When a chemical expires or reaches the retest date, the chemical manufacturer may be contacted to determine if the expiration date has been extended. If this is the case, the updated expiration date will be written or printed on the chemical container(s) along with the analyst initials and date.

If a chemical manufacturer has not provided a retest or expiration date on the chemical container or associated documentation, an expiration date will be assigned by the laboratory according to the nature of the material. This expiration date will be written or printed onto the chemical container(s) along with the analyst initials and date.

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General expiration date guidelines are:

Liquid acids/bases	2 years from the date received
Organic solvents	2 years from the date received
Solid acids/bases	5 years from the date received
Other solid chemicals	10 years from the date received
Other liquid chemicals	5 years from the date received

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## Evidence Handling

Biological evidence is refrigerated except during analysis or transport. Suspected beverage alcohol samples are not required to be refrigerated. All unsealed 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.

**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.

Appropriate personal protective equipment (PPE) for work in the toxicology laboratory is, at a minimum, gloves and a laboratory coat when documenting evidence packaging and gloves, laboratory coat, eye protection, and face mask or face shield when diluting samples.

## Item Selection and Sampling

### Item Selection

Not all items submitted to the laboratory for analysis will routinely be analyzed. The number of samples analyzed will be kept to a reasonable number and limited to relevant items based on information conveyed to the laboratory by law enforcement and/or a prosecutor. Once probative results are obtained, additional analysis will not be performed. If no information is provided to the laboratory by law enforcement and/or a prosecutor to guide sample selection, the following guidelines are to be utilized:

#### Blood Alcohol

The Blood and Breath Alcohol Program views blood alcohol results and evidentiary breath test results as equally valid in determining a subject's ethanol level. For this reason, samples submitted for blood alcohol testing that have a completed evidentiary breath result will not be analyzed for blood alcohol content without further information from law enforcement and/or the prosecutor about the need for additional testing.

When multiple blood specimens from the same subject are submitted, the analyst will choose one for testing. Samples that have the earliest collection times and that are collected in tubes containing anticoagulant and/or preservative are preferred.

#### Beverage Alcohol

Liquids that are inside manufacturer sealed containers listed as containing ethanol will not be tested. When multiple containers of suspected beverage alcohol are submitted, the analyst will choose one for testing.

Testing of additional containers may occur in the following circumstances:

- The contents from the initially selected container tested negative
- The combined volume (obtained from the manufacturer labels) of multiple containers exceeds a legal threshold listed in AS 04.16.200. These critical volumes are:

Distilled spirits:	10.5 liters
Wine:	24 liters
Malt beverages:	12 gallons

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### 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 may often have multiple units collected and submitted under the same item number. 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 (or similar):

**"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, 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 is 95% confidence that at least 90% of the units contain the analyte. The sampling plan employed is outlined in the Hypergeometric Sampling Plan appendix. 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 in the report.

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## Analysis Procedure

### Instrument Preparation

Each day, prior to running a sequence, the mass spectrometer is calibrated using the Autotune program. Successful calibration indicates that the instrument assigned the proper masses to the Perfluorotributylamine (PFTBA) calibration standard. A Tune Evaluation is performed and all expected results list OK prior to use of the instrument. A copy of the Autotune report and tune evaluation is included in the control packet associated with the run.

**Check gas supplies (helium, hydrogen, zero air) to ensure sufficient amounts before starting a sequence.**

### Sample Preparation

The maximum number of case samples per run is 15. Two successive runs may be performed on one instrument if each run is bracketed by all appropriate calibrators and controls. Standards, controls, and case samples must warm to room temperature prior to sampling. Ensure the lot numbers and expiration dates of the controls and standards being used are reflected accurately in the Ethanol Control Worksheet.

Prepare two vials for each sample and control that is to be analyzed. Set up one vial each for the negative controls, mixed volatiles standard, and calibration standards. A negative control is run as the first sample in each sequence followed by the calibration standards. A negative control and the mixed volatiles standard are run following the calibration standards. A 0.300 aqueous ethanol control, whole blood and/or beverage control and a 0.025 aqueous ethanol control are run in duplicate immediately prior to and following a batch of case samples. The calibration standards are then sampled and analyzed a second time at the end of the sequence. The averages responses from the two sets of calibration standards are used to create the calibration curve. An example blood alcohol sequence can be found in the Sequence Template appendix.

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 or tubing. 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. Label the headspace vial for identification. Dispense 1000  $\mu\text{L}$  of internal standard diluent, along with 100  $\mu\text{L}$  of sample, standard, or control into the headspace vials using the diluter/dispenser. Wipe the tip of the dispenser between each sample. Cover the vials with caps and crimp tightly onto the vials. Continue this process for all samples in the run.

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More than one analyst may include samples in a single run, but each analyst must dilute his/her own case samples and the samples must be diluted in the order they will be analyzed. For more information see Combined Analyst Runs.

Suspected beverage alcohol samples can be run in separate batches or combined with 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. In a combination blood and beverage run, both the whole blood control and the beverage control are run before and after the case samples. The beverage control is diluted as described below.

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.

In batches that include beverage samples, the negative control will also have a preliminary dilution to ensure no contamination is present in the pre-dilution step.

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.

### Reprocessing Data

When analysis is complete, the sequence is reprocessed with the standards treated as samples to ensure averaged responses from the two sets of calibration standards are used to create the calibration curve. Example blood alcohol sequences for analyzing and reprocessing samples can be found in the Sequence Template appendix.

### Passing Criteria – Ethanol Quantitation

Upon completion of the run, verify that:

- Negative controls demonstrate no measurable ethanol
- The mixed volatiles standard has baseline separation of all components and that the mass spectrum of ethanol contains the ions 31, 45, and 46
- Averaged calibrator responses were used for calibration curve
- Calibration standards results (after reprocessing) are within  $\pm 0.005$  or 5%, whichever is greater, of their expected value
- The RPD for all aqueous, whole blood, and beverage controls is 3.0% (rounded to one decimal place) or less when entered in the Ethanol Control Worksheet.
- All aqueous and whole blood control averages fall within the passing range when entered in the Ethanol Control Worksheet.

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- All beverage control averages fall within the passing when entered in the Ethanol Control Worksheet – Beverage.

If a negative control, mixed volatiles standard, 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.

### Control Packs

#### When to Create a Control Pack

Control packs are generated for each run that is completed, even if the run fails (examples: calibrator or control failure). Control packs are not generated for runs that are not completed (examples: MS filament burnt out, autosampler error, power failure, etc.).

#### Control Pack Contents

Each control pack contains the following:

- The Autotune report and Tune Evaluation report
- A copy of the sequence table for the run
- Ethanol calibration curve
- The ETOH custom report for each negative control, calibrator, positive control (aqueous, blood and/or beverage), and mixed volatiles control analyzed
- Ethanol Control Worksheet (Blood, Beverage, or Both for Combined Runs)

#### *Matrix-Matched Control Failure*

If a matrix-matched control causes a portion of a run to fail (e.g., whole blood control fails on a combined run), the case samples matching the failing control type also fail. However, case samples of other types may pass if all other calibrators and controls are within specification. If this occurs, a failing control pack (marked -F) will be created for the failing sample type and a separate passing control pack will be created for the passing sample type. The respective control packs need only include the Ethanol Control Worksheet and matrix-matched control instrument reports for the corresponding sample type; however, all other elements listed in [Control Pack Contents](#) must be included in both control packs.

#### Control Pack Identification

Control packs are named with a control pack identifier. The control pack identifier is stamped on each page of the control pack using the format YYYYMMDDINT where INT is the analyst's initials. Control pack identifiers for failed runs will be in the format YYYYMMDDINT-F.

#### Control Pack Storage

All generated Control Packs are stored electronically on the laboratory's SharePoint document library.

### Evaluation of Case Sample Data

Custom reports are generated for each vial run in a completed sequence. The case sample reports are evaluated by the analyst to ensure:

- Duplicate analyzed values of a case sample have an RPD of 3.0% (rounded to one decimal place) or less.
- The mass spectra of samples with positive ethanol results contain the ions 31, 45 and 46 (see Ethanol Identification) for more information.
- Any significant peaks present other than ethanol or n-propanol are evaluated for qualitative analysis (see Minimum Criteria for Identification (Qualitative Analysis)) and documentation of the mass spectrum is retained in the case file.
- If the duplicate analyzed values of a case sample have an RPD greater than 3.0% and the average of the values is 0.020 g/100mL or greater, the sample will be re-diluted and reanalyzed up to two more times. If the third dilution fails, the sample will be reported as insufficient for quantitation (see Reporting Language section).

If a run does not complete (examples: MS filament burnt out, autosampler error, power failure, etc.), the case sample data for this run are not retained. If a run completes and fails (examples: calibrator or control failure), the case sample data from this run are retained in the case files.

### Minimum Criteria for Identification (Qualitative Analysis)

The minimum criteria for the identification of a volatile substance are a positive retention time comparison and a positive mass spectral comparison on two separate samplings of an item.

### Ethanol Identification

The ethanol retention time is determined by the GC method. The sequence is designed to set the retention time of ethanol to that of the first ethanol calibrator. Subsequent peaks must fall within  $\pm 0.1$  minute of that retention time. 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 in the ETOH custom report. The analyst will ensure that the mass spectra of samples with positive ethanol results contain the ions 31, 45 and 46.

### Other Volatile Identification

When reviewing the custom report generated for ethanol quantitation the analyst may observe the presence of other volatile substances that can be found in biological or beverage specimens. Volatiles other than ethanol will not be identified.

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### **Technical Deviations from Analysis Procedure**

Pre-approved deviations from technical procedures (not specifically permitted by this procedure manual or a related Working Instruction) will follow the Deviation Request Form Policy in the Quality Assurance Manual.

Technical deviations that are not pre-approved are addressed in the Quality Assurance Manual under the nonconforming work policy. Quality Assurance Reviews for blood alcohol testing are provided to the Scientific Director prior to being forwarded to the Quality Assurance Manager for final review.

If a technical deviation occurs in casework performed by the Scientific Director, follow the same Deviation Request and Quality Assurance Review processes as other analysts. The approving authority for a Deviation Request by the Scientific Director will be another competent analyst in the blood alcohol discipline.

Technical deviations are reviewed by another competent analyst during case review.

## Reporting Language

Approved report language is listed below. Any alternative wording on reports is not permitted without prior approval from the discipline supervisor. This approval must be documented in the case file. For more information on report content see Reports.

For blood ethanol quantitation, the concentration will be reported as follows:

- Ethanol values less than or equal to 0.009 g/100mL: **“None Detected”**
- Ethanol values greater than or equal to 0.010 and less than 0.020 g/100mL: **“Less than 0.020 g/100mL”**
- Ethanol 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 ethanol values over 0.500 g/100mL will be diluted and reanalyzed.

For beverage ethanol quantitation, the mean of the duplicates (truncated to 3 decimal places) is multiplied by the dilution used and then converted to volume of ethanol per volume of liquid (%v/v) by dividing by the density of ethanol. For example:

$$\frac{0.050 \frac{\text{g EtOH}}{100 \text{ mL liquid}} \times 100 (\text{Dilution factor, unitless})}{0.789 \frac{\text{g EtOH}}{\text{mL EtOH}} (\text{Density})} = \frac{6.3 \text{ mL EtOH}}{100 \text{ mL liquid}} = 6.3\% \text{ v/v}$$

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 no dilution. The beverage concentration will be reported as follows:

- If less than 0.012% v/v: **“None Detected”**
- 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 greater than or equal to 0.025% v/v: report ethanol concentration.
- 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 = Truncated to 1 decimal place
  - 10 times pre-dilution = Truncated to 2 decimal places
  - No pre-dilution = Truncated to 3 decimal places

For factory-sealed beverage samples that also request latent print analysis, the analyst may issue a report based on a review of the latent print analyst’s report and notes indicating the condition of the factory seal.

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- In this instance the Item Description should indicate **“Not retrieved and/or not analyzed”** (see the JusticeTrax LIMS-Plus 3.8 manual for more information on populating this field.)
- The result should read **“Not Analyzed.”**
- The packaging dropdown **“Not Retrieved”** should be selected.
- The notes should read **“Item was not retrieved or opened. Item description was taken from the Latent Print Processing report and notes completed on [Date of Latent Print Report]. Description from notes indicates [Description of Item from Latent Print Report]. Notes also indicate the container(s) have intact factory seals.”**

For samples where sample quality prohibits preparation due to diluter clogging or samples where duplicate analyzed values of a case sample have an RPD of greater than 3.0% on three dilutions and the ethanol value is greater than 0.020 g/100ml, the sample will be reported as: **“Sample quality insufficient for quantitation.”**

When drug toxicology testing is requested in addition to alcohol testing, the following supplemental statements are utilized in the report. See the [Drug Toxicology Testing Requests](#) section for more information on when samples should be forwarded on for drug toxicology testing.

- **“Drug analysis to follow.”**
- **“It is the policy of the Crime Lab to not have drug analysis performed if the blood alcohol concentration is at or above 0.100 g/100 mL. If you require further testing please contact the Toxicology Section.”**
- **“The Alaska Crime Lab does not currently send evidence in non-driving offenses for drug toxicology testing. If you require further testing you will need to contact an outside laboratory. The item of evidence is being returned to your agency.”**

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 and will be rounded to the same number of decimal places. For additional information regarding measurement of uncertainty, see [Uncertainty of Measurement](#).

## Administrative

### Reports

A report will be issued for each case analyzed. Each report will clearly communicate the items received and analyzed. The quality manual outlines the common requirements for laboratory reports. The forensic alcohol discipline utilizes three different request types depending on the sample matrix being tested: Blood Alcohol, Beverage Alcohol, and Serum/Plasma. The report templates are tied to the relevant request type in the LIMS and the analyst notes are integrated into the report templates. In addition to the common requirements in the quality manual, forensic alcohol reports will include the following:

- A brief description of the item(s) analyzed
- Ethanol result for each item analyzed with applicable uncertainty
- Whole blood equivalent converted ethanol result (for Serum/Plasma)
- Any additional volatiles identified for each item
- Sampling plan, if used
- Information on any additional toxicology testing requested

When the analyst determines the report is complete and ready to send on for review, the completion date and the analyst's electronic signature will be applied.

Prior to forwarding the case on for review, the analyst will cancel any Drug Toxicology requests that are triaged due to blood alcohol results greater than 0.100 g/100mL.

### Technical Record Requirements

The Blood Alcohol technical record encompasses the laboratory report as well as the instrument data and control pack. The technical record will include, at a minimum:

- The start and end date of analysis
- A detailed description of each items' packaging and contents. This must include enough detail to properly convey the information to the technical and administrative reviewer.
- The number and type of tubes submitted
- Any unusual aspects of the sample
- Date and time of collection (if available)
- Subject name (if available)
- Any significant discrepancies or broken seals
- The Control Pack Identifier(s) for all completed runs that include the item, including completed runs that failed
- If a sample was analyzed on a failed run (complete or incomplete), the reason for the failure will be stated

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- Documentation of the occurrence of any incomplete runs that include the item.
- Results for each duplicate, calculated average, and calculated RPD
- The dates of each Lab Activity (refer to [Appendix 1 - Lab Activity Dates](#))

### Amendments to Technical Records

For elements of the technical record not entered into JusticeTrax fields (e.g., instrument printouts, control pack), amendments to these documents after any associated requests have been turned in for technical review must be made in such a way that the original data is retained, the altered aspects are clear, the person responsible for the alteration is identified, and the date on which the amendment was made is present.

One example of this is including a new copy of the Ethanol Control Worksheet if it needed to be regenerated due to a correction. The original Worksheet should be marked in such a way as to be clear that it was not used. The corrected worksheet will specify why regenerating the worksheet was necessary as well as the person responsible and date of the action. Another example is a PDF correction on an instrument printout that lines through the incorrect case number, clearly states the correct case number along with the date and initials of the person making the correction.

### Reviews

The evidence must be returned to the appropriate evidence return location or another laboratory member prior to administrative review. Each case will be technically and administratively reviewed prior to distribution. The minimum requirements listed in the Quality Assurance Manual will be completed. The discipline supervisor will be consulted on any issues between the case analyst and the technical reviewer and will make the final decision.

The technical/administrative reviewer is responsible for ensuring items that require drug toxicology testing are ready to be forwarded to the Public Health Lab. After the technical and administrative reviews are completed, the reviewer should un-pend the drug toxicology request and add a copy of the case information to the electronic storage file for the Public Health Lab.

### Release of Preliminary Results

Preliminary results may be provided by the case analyst when necessary. This communication will be documented in the case file and will include a disclaimer that this is preliminary information. The Quality Assurance Manual outlines further details on the release of preliminary results under 7.8.1.2.1.

### Amended Reports

An amended report will be issued when an error is discovered after the report has been distributed. The case analyst will notify the supervisor when an amended report is issued. Refer to the Quality Assurance Manual and JusticeTrax manual for required reporting language and instructions when issuing an amended report.

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### Discovery

Discovery for the Toxicology discipline is managed by the discipline supervisor other than routine bench note requests. For more information on discovery request storage, refer to the JusticeTrax LIMS-Plus 3.8 Manual.

### Forensic Alcohol Opinion Reports

Analysts in the Toxicology discipline are often asked to provide written pretrial opinions based on facts of the case as well as the final blood alcohol result. These opinions are documented in the LIMS using the request type Forensic Alcohol Opinion. In the opinion report the analyst will document all information provided that was utilized to form the opinion along with any assumptions being made. The opinion request is technically reviewed by another competent analyst.

### Ethanol Control Worksheet

#### Overview

The Ethanol Control Worksheet and Ethanol Control Worksheet-Beverage are separately controlled documents which contains current mixed volatile standard, calibration standard, and control lot information, control passing ranges, and identification and current use of diluter/dispensers. They are programmed to calculate whether control data from a particular analysis sequence meets the acceptance criteria outlined in the Passing Criteria – Ethanol Quantitation. The Ethanol Control Worksheets are also used to track all control data for future estimations of measurement uncertainty. The following paragraphs describe how data entry and calculations are performed in the Ethanol Control Worksheets.

#### Ethanol Control Worksheet Calculations

All aqueous, whole blood, and beverage controls are run in duplicate. 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 3.0% (rounded to one decimal place) 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  $\pm 0.005$  g/100mL or 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, whichever is greater.

For beverage controls the average truncated to 3 decimal places is converted to alcohol by volume and is compared to the expected value. The passing range is equal to the expected value  $\pm 0.006$ , 0.06, or 0.6% v/v or the beverage alcohol method's current expanded combined standard uncertainty at 95% coverage converted to % v/v units and rounded to one decimal place whichever is greater.

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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.

### Combined Analyst Runs

In a combined analyst alcohol run, the primary analyst dilutes and prepares all samples except for the second analyst's samples. The second analyst must dilute their samples, in order, at the appropriate point in sample dilution.

The primary analyst will set up the instrument and sequence and will be listed as the operator of the instrument. The primary analyst will reprocess the run, evaluate the control pack data, and prepare the control pack. The second analyst is responsible for evaluating the control pack and their own casework data. The second analyst will add their date of interpretation and initials to the Ethanol Control Worksheet in the control pack. The initials of the second analyst will appear in the custom reports for each sample prior to entering the data into LIMS.

The primary analyst may not technically review the second analyst's work because they created the initial control pack. The second analyst may technically review the control pack as well as the primary analyst's samples.

### Drug Toxicology Testing Requests

Outsourced toxicology services are currently funded through a National Highway Traffic Safety Administration (NHTSA) grant managed by the Alaska State Public Health Laboratory. The Alaska Scientific Crime Detection Laboratory does not currently send evidence from non-driving related cases out for drug toxicology analysis.

Cases arriving at the laboratory for Toxicology testing will be screened by a member of the Chemistry section. The screener will review all documents accompanying the case and look for any associated breath records. Evidence will be sent for drug toxicology analysis if the case meets one or both of the following criteria:

- The submitting agency requests a drug toxicology analysis and the case involves a driving-related incident leading to the serious injury or death of a person other than the driver.
- The submitting agency requests a drug toxicology analysis, the case involves a driving-related offense, and the blood or breath alcohol 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 out for drug toxicology analysis when grant funding allows.

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To ensure all forensic alcohol requests are complete prior to evidence being forwarded for drug toxicology testing, samples will not be forwarded to the Public Health Lab until the technical/administrative reviewer indicates that the case is complete.

### Continuing Education of Toxicology Analysts

Toxicology analysts are required to engage in continuing education to stay up-to-date on topics such as new technologies, instrumentation, and forensic standards of practice. Other terms used to refer to continuing education may include training and professional development.

Each analyst should average 16 hours per year of continuing education over a 4-year cycle. This may be in person with an instructor, distance learning, or computer-based. Additional requirements for continuing education are as follows:

- Continuing education shall be relevant to the laboratory's mission
  - If it is unclear whether an available training or continuing education opportunity meets this requirement, please consult with the Technical Lead and/or discipline supervisor
- Continuing education shall be documented in JusticeTrax and must include one or more of the following:
  - Literature review documentation (see Literature Review Requirements)
  - Issuance of a certificate of completion or diploma
  - Publishing a paper
  - Verification of attendance
  - Recording of a presentation or exercise
- Continuing education may be obtained from a variety of sources including, but not limited to, the following:
  - Chemistry or instrumental courses taught at the postsecondary educational level (i.e. college or university)
  - Instrument operation or maintenance courses taught by vendors
  - In-service classes conducted by employers
  - Webinars from academic or forensic organizations
  - Current literature review (see Literature Review Requirements)
- Literature Review Requirements: Literature review may be counted towards continuing education requirements if one of the following criteria are met:
  - Read an article and participate in a group discussion with other lab staff:
    - Meeting minutes showing attendance will be stored in the individual training record in JusticeTrax
    - Up to one hour of continuing education credit may be claimed for completion
  - Read an article then write an assessment of the article content:
    - An assessment may include, but is not limited to:
      - Considering the pros and cons of the information presented

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- Developing an informed opinion as to whether the practice/procedure would be beneficial to the laboratory
- Identifying limitations or alternatives
- The written assessment will be stored in JusticeTrax as documentation of the literature review
- Up to one hour of continuing education credit may be claimed for completion

### Performance Monitoring Plan

The ANAB Scope of Accreditation to ISO/IEC 1725:2017 lists the Key Equipment and Technologies used within the Toxicology discipline along with the listed components of analysis. The Toxicology discipline monitors the performance of these techniques by proficient toxicology analysts through the performance monitoring plan.

At least once per accreditation cycle, the performance of each component of the Scope of Accreditation will be monitored for each proficient analyst. Annually, prior to the annual management review, the Toxicology Technical Lead will review the previous year's performance monitoring plan to ensure all components and technologies are being addressed and submit an updated plan.

The performance monitoring plans are stored in the quality assurance records.

### Proficiency Testing

#### Blood Alcohol

The guideline for satisfactory completion is based on the manufacturer's expected results and the average of all ANAB respondents (if ANAB respondents are not listed then all respondents will be used). This means  $\pm 2$  standard deviations of the average of all ANAB respondents for samples. The current reported uncertainty of measurement range may 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.

#### Beverage Alcohol

The guideline for satisfactory completion is based on the manufacturer's expected results and the average of all ANAB respondents (if ANAB respondents are not listed then all respondents will be used). This means  $\pm 2$  standard deviations or  $|z| \leq 2$  of the average of all ANAB respondents (or all 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.

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Beverage 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 chemistry supervisor will destroy the samples after the results have been received from the proficiency test provider.

### Extended Absence Policy

When an analyst is away from the laboratory for an extended period (six months or longer), he/she will be required to successfully complete an internal competency test before resuming casework analysis. The scope of the competency test and authorization to resume casework are the responsibility of the chemistry supervisor.

### Forensic Alcohol Kit Dissemination

The crime lab provides blood alcohol collection kits and beverage alcohol collection kits to Alaska law enforcement agencies.

The distribution and tracking of these kits are handled by DPS Supply. All requests for blood or beverage alcohol collection kits should be emailed to DPS supply at [DPS.supply.orders@alaska.gov](mailto:DPS.supply.orders@alaska.gov).

Whenever possible, the following information should be provided:

- Item and quantity requested
- Whether it needs to be mailed or will be picked up
- Contact name
- Phone number
- Mailing address

### Key Control

Each analyst has a padlock with a unique key that is used to secure evidence. Top Management has access to the master key to these padlocks.

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

## 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" dated December 20, 2013. The initial report and updates are stored on the laboratory SharePoint document library.

The initial report addresses the following elements:

- Statement of the measurand (liquid alcohol concentration in g/100 mL)
- 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 (k-value), the coverage probability, and the resulting expanded uncertainty
- The schedule to review and/or recalculate the measurement of uncertainty.

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](#) located in the laboratory SharePoint document library.

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

**The current expanded combined standard uncertainty at 95% coverage for blood ethanol analysis is  $\pm 0.005$  g/100 mL or 4.8% of the reported value (whichever is greater). For beverage alcohol analysis, it is  $\pm 0.6$ , 0.06, or 0.006 %v/v (depending on dilution factor) or 4.7% of the reported value (whichever is greater).**

The blood ethanol expanded combined standard uncertainty also applies to the verification of Calibration Standards and Aqueous Ethanol Controls as defined in this manual.

## Hypergeometric Sampling Plan<sup>1</sup>

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.

Deviations from the sampling plan require prior approval from the Technical Lead and/or discipline supervisor and will be documented in LIMS.

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<sup>1</sup> Information is taken from the European Network of Forensic Science Institutes (ENFSI) Guidelines on Sampling of Illicit Drugs for Qualitative Analysis (<http://enfsi.eu/documents/forensic-guidelines/>) A copy of the 2016 edition is located in the laboratory SharePoint library ([Blood Alcohol Required Reading](#)).

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### Microlab 500 Dilution Methods

<b>Method: ETHANOL</b>	Left	Right
Syringe Size (uL)	1000	100
Ratio	1:10.0	
Dilution	1/11.0	
Diluent Volume (uL)	1000.0	
Air Gap Volume (uL)		5.0
Sample Volume (uL)		100.0
Final Volume (uL)	1100.0	
Syringe Fill Speed	5	
Syringe Aspirate Speed		2
Syringe Dispense Speed	6	2
Syringe Fill Mode	AUTO	
Air Gap Mode	AUTO	
Air Gap Delay	0.3	
Wash Volume (uL)	1000.0	
Syringe Fill Speed	3	
Syringe Dispense Speed	6	

<b>Method: B100</b>	Left	Right
Syringe Size (uL)	5000	100
Ratio	1:99.0	
Dilution	1/100.0	
Diluent Volume (uL)	4950.0	
Air Gap Volume (uL)		5.0
Sample Volume (uL)		50.0
Final Volume (uL)	5000.0	
Syringe Fill Speed	5	
Syringe Aspirate Speed		2
Syringe Dispense Speed	5	2
Syringe Fill Mode	AUTO	
Air Gap Mode	AUTO	
Air Gap Delay	0.3	
Wash Volume (uL)	5000.0	
Syringe Fill Speed	5	
Syringe Dispense Speed	5	

<b>Method: B10</b>	Left	Right
Syringe Size (uL)	1000	100
Ratio	1:9.0	
Dilution	1/10.0	
Diluent Volume (uL)	900.0	
Air Gap Volume (uL)		5.0
Sample Volume (uL)		100.0
Final Volume (uL)	1000.0	
Syringe Fill Speed	3	
Syringe Aspirate Speed		2
Syringe Dispense Speed	3	2
Syringe Fill Mode	AUTO	
Air Gap Mode	AUTO	
Air Gap Delay	0.3	
Wash Volume (uL)	1000.0	
Syringe Fill Speed	3	
Syringe Dispense Speed	3	

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**Microlab 600 Dilution Methods**

<b>Method: Ethanol</b>					
#	Trigger	Left Valve	Left Volume (uL)	Right Valve	Right Volume (uL)
1	Auto	IN	1000	OUT	0
2	Auto	OUT	0	OUT	5
3	Probe	OUT	0	OUT	100
4	Probe	OUT	-1000	OUT	-105
5	Auto	IN	1000	OUT	0
6	Probe	OUT	-1000	OUT	0
		Syringe Speed	Left = 200 uL/s	Right = 50 uL/s	
		Syringe Size	Left = 1000 uL	Right = 100 uL	

<b>Method: B100</b>					
#	Trigger	Left Valve	Left Volume (uL)	Right Valve	Right Volume (uL)
1	Auto	IN	4950	OUT	0
2	Auto	OUT	0	OUT	5
3	Probe	OUT	0	OUT	50
4	Probe	OUT	-4950	OUT	-55
5	Auto	IN	5000	OUT	0
6	Probe	OUT	-5000	OUT	0
		Syringe Speed	Left = 1250 uL/s	Right = 50 uL/s	
		Syringe Size	Left = 5000 uL	Right = 100 uL	

<b>Method: B10</b>					
#	Trigger	Left Valve	Left Volume (uL)	Right Valve	Right Volume (uL)
1	Auto	IN	900	OUT	0
2	Auto	OUT	0	OUT	5
3	Probe	OUT	0	OUT	100
4	Probe	OUT	-900	OUT	-105
5	Auto	IN	1000	OUT	0
6	Probe	OUT	-1000	OUT	0
		Syringe Speed	Left = 200 uL/s	Right = 50 uL/s	
		Syringe Size	Left = 1000 uL	Right = 100 uL	

# Alaska Scientific Crime Detection Laboratory

## Ethanol Quantitation Procedure Manual

Effective: 6/3/2024

Version: 8.0

### Sequence Template

#### Analyzing:

Sample Log Table														
Data Path: C:\MSDCHEM\11302017			Browse...						Method Path: C:\MSDCHEM\1\METHODS			Browse...		
	Type	Vial	Sample	Method / Keyword	Data File	Level	Update RF	Update RT						
1	Sample	1	Blank	ETHANOL	11302017COB_001		No Update	No Update						
2	Calibration	2	0.020 STD	ETHANOL	11302017COB_002	20	Replace	Replace						
3	Calibration	3	0.100 STD	ETHANOL	11302017COB_003	100	Replace	No Update						
4	Calibration	4	0.200 STD	ETHANOL	11302017COB_004	200	Replace	No Update						
5	Calibration	5	0.500 STD	ETHANOL	11302017COB_005	500	Replace	No Update						
6	Sample	6	Blank	ETHANOL	11302017COB_006		No Update	No Update						
7	Sample	7	Mixed Volatiles	ETHANOL	11302017COB_007		No Update	No Update						
8	Sample	8	0.300 Control--	ETHANOL	11302017COB_008		No Update	No Update						
9	Sample	9	0.300 Control--	ETHANOL	11302017COB_009		No Update	No Update						
10	Sample	10	WBC--	ETHANOL	11302017COB_010		No Update	No Update						
11	Sample	11	WBC--	ETHANOL	11302017COB_011		No Update	No Update						
12	Sample	12	0.025 Control--	ETHANOL	11302017COB_012		No Update	No Update						
13	Sample	13	0.025 Control--	ETHANOL	11302017COB_013		No Update	No Update						
14	Sample	14	Sample 1	ETHANOL	11302017COB_014		No Update	No Update						
15	Sample	15	Sample 1	ETHANOL	11302017COB_015		No Update	No Update						
16	Sample	16	Sample 2	ETHANOL	11302017COB_016		No Update	No Update						
17	Sample	17	Sample 2	ETHANOL	11302017COB_017		No Update	No Update						
18	Sample	18	0.300 Control--	ETHANOL	11302017COB_018		No Update	No Update						
19	Sample	19	0.300 Control--	ETHANOL	11302017COB_019		No Update	No Update						
20	Sample	20	WBC--	ETHANOL	11302017COB_020		No Update	No Update						
21	Sample	21	WBC--	ETHANOL	11302017COB_021		No Update	No Update						
22	Sample	22	0.025 Control--	ETHANOL	11302017COB_022		No Update	No Update						
23	Sample	23	0.025 Control--	ETHANOL	11302017COB_023		No Update	No Update						
24	Calibration	24	0.020 STD	ETHANOL	11302017COB_024	20	Average	No Update						
25	Calibration	25	0.100 STD	ETHANOL	11302017COB_025	100	Average	No Update						
26	Calibration	26	0.200 STD	ETHANOL	11302017COB_026	200	Average	No Update						
27	Calibration	27	0.500 STD	ETHANOL	11302017COB_027	500	Average	No Update						
28														

#### Reprocessing:

Sample Log Table														
Data Path: C:\MSDCHEM\11302017			Browse...						Method Path: C:\MSDCHEM\1\METHODS			Browse...		
	Type	Vial	Sample	Method / Keyword	Data File	Level	Update RF	Update RT						
1	Sample	1	Blank	ETHANOL	11302017COB_001		No Update	No Update						
2	Sample	2	0.020 STD	ETHANOL	11302017COB_002	20	Replace	Replace						
3	Sample	3	0.100 STD	ETHANOL	11302017COB_003	100	Replace	No Update						
4	Sample	4	0.200 STD	ETHANOL	11302017COB_004	200	Replace	No Update						
5	Sample	5	0.500 STD	ETHANOL	11302017COB_005	500	Replace	No Update						
6	Sample	6	Blank	ETHANOL	11302017COB_006		No Update	No Update						
7	Sample	7	Mixed Volatiles	ETHANOL	11302017COB_007		No Update	No Update						
8	Sample	8	0.300 Control--	ETHANOL	11302017COB_008		No Update	No Update						
9	Sample	9	0.300 Control--	ETHANOL	11302017COB_009		No Update	No Update						
10	Sample	10	WBC--	ETHANOL	11302017COB_010		No Update	No Update						
11	Sample	11	WBC--	ETHANOL	11302017COB_011		No Update	No Update						
12	Sample	12	0.025 Control--	ETHANOL	11302017COB_012		No Update	No Update						
13	Sample	13	0.025 Control--	ETHANOL	11302017COB_013		No Update	No Update						
14	Sample	14	Sample 1	ETHANOL	11302017COB_014		No Update	No Update						
15	Sample	15	Sample 1	ETHANOL	11302017COB_015		No Update	No Update						
16	Sample	16	Sample 2	ETHANOL	11302017COB_016		No Update	No Update						
17	Sample	17	Sample 2	ETHANOL	11302017COB_017		No Update	No Update						
18	Sample	18	0.300 Control--	ETHANOL	11302017COB_018		No Update	No Update						
19	Sample	19	0.300 Control--	ETHANOL	11302017COB_019		No Update	No Update						
20	Sample	20	WBC--	ETHANOL	11302017COB_020		No Update	No Update						
21	Sample	21	WBC--	ETHANOL	11302017COB_021		No Update	No Update						
22	Sample	22	0.025 Control--	ETHANOL	11302017COB_022		No Update	No Update						
23	Sample	23	0.025 Control--	ETHANOL	11302017COB_023		No Update	No Update						
24	Sample	24	0.020 STD	ETHANOL	11302017COB_024	20	Average	No Update						
25	Sample	25	0.100 STD	ETHANOL	11302017COB_025	100	Average	No Update						
26	Sample	26	0.200 STD	ETHANOL	11302017COB_026	200	Average	No Update						
27	Sample	27	0.500 STD	ETHANOL	11302017COB_027	500	Average	No Update						
28														

## Appendix 1 - Lab Activity Dates

The following lab activities are conducted during Blood Alcohol testing. The analyst records the dates of each lab activity in the technical record. The technical record is composed of the laboratory report as well as the instrumental data attachments and Ethanol Control Worksheet. This table lists the location where the lab activity dates are recorded and is intended to be used as a guide for the analyst and technical reviewer.

Activity Name	Location of Date
Sample Dilution**	Worksheet section of report
Instrument Test Dates	Instrument Printouts
Control Pack Evaluation	Ethanol Control Worksheet in Control Pack
Data Interpretation	Worksheet section of report
Beverage Pre-Dilution	Ethanol Control Worksheet in Control Pack

\*\*The Sample Dilution date is defined as the date the run was initiated by default. If the Sample Dilution date is NOT the same as the run initiation date, a note will be added in the worksheet section of the report.\*\*

**NOTE:** Some lab activities may have more than one date if they are performed more than once or, for example, instrumental data are interpreted on different days.

# Alaska Scientific Crime Detection Laboratory

## Ethanol Quantitation Procedure Manual

Effective: 6/3/2024

Version: 8.0

### Revision History

Location	Revision made
All	Fixed formatting
Table of Contents	Updated
Header	Updated name of manual from "Ethanol Quantitation and Volatile Analysis Procedure Manual" to "Ethanol Quantitation Procedure Manual".
<a href="#">Other Volatile Identification</a>	Rewrote section. Removed sub sections Retention Time Comparison, Mass Spectral Interpretation, and Documentation for Volatiles other than Ethanol.
<a href="#">Reporting Language</a>	Removed "Volatiles that are identified, but not quantitated, will be reported as _____ identified but concentration not determined."
<a href="#">Digitally Archiving Verification Records</a>	Expanded controls to be archived into each control type (aqueous, whole blood and beverage). Added a timeframe for digital archiving (annually).
<a href="#">Combined Analyst Runs</a>	Added "The second analyst will add their date of interpretation and initials to the Ethanol Control Worksheet in the control pack." Updated "The initials of the second analyst will appear in the custom reports for each sample prior to entering the data into LIMS."