Blood Alcohol Analysis

1 GENERAL PRINCIPLE

This procedure outlines a simple method for the detection and quantitation of ethanol in blood samples by “Headspace” gas chromatographic procedure. Static headspace offers a reliable, simple, and accurate way to quantitate volatile compounds in a variety of liquid matrices. Practically, the sample and an internal standard are added to a vial and the vial is sealed. The sealed vial is then placed into the heated sample carousel of the Headspace Analyzer. The increased temperature causes the volatiles to be released from the solution into the “headspace” above the liquid. The headspace is then sampled and analyzed for the presence of the targeted analytes via dual column gas chromatography (GC) equipped with dual flame ionization detectors (FID) and quantified.

g% = g/dL; e.g. 0.08 g% = 0.08 g of alcohol in 100mL of blood is the same as 0.08 g/dL

1.1 Analytes Detected

Methanol, Ethyl Alcohol, Isopropanol, and Acetone. N-propanol as an internal standard.

1.2 Definitions:

Alcohol – Any substance containing Ethanol, Methanol, Propanol, or Isopropanol. N.C.G.S. 20-4.01 (1a).

Alcohol Concentration – the concentration of Alcohol in a person, expressed as: grams of alcohol per 100 milliliters of blood. N.C.G.S. 20-4.01 (1b) a.

Chemical Analyst – A person granted a permit by the Department of Health and Human Services under G.S 20-139.1 to perform chemical analyses. N.C.G.S. 20-4.01(3b).

1.3 Controls and Calibrators

1.3.1 Calibrators and controls used to calculate uncertainty of measurement will be purchased or prepared using certified reference materials from ISO 17025 and ISO 17034 accredited vendors, when available. Such vendors include Cerilliant, Restek, and Lipomed.

1.3.2 Non-calibrator solutions or controls not used in the uncertainty of measurement calculation may be purchased or prepared from other accredited vendors, when available. Such vendors include UTAK or Fisher.

1.3.3 0.05% n-Propanol in water (Internal Standard):
To a 1 L volumetric flask, add 0.50 mL (500 µL) of n-Propanol. Fill to the mark with DI water. Mix well and transfer to a reagent bottle. Label the bottle with the name of the reagent, date of preparation, lot number (the date in numeric format followed by the initials of the preparer), and expiration date. Lot number example - 092016MJ. The internal standard solution can be prepared in any amount provided the components’ ratio is kept consistent.
Storage - Room temperature Expiration - 1 year

1.3.4 Ethyl Alcohol Calibrators [Cerilliant/Restek or equivalent]:
0.05 g%, 0.10 g%, 0.30 g% and 0.40 g%
Storage- Refrigerator Expiration- as labeled

1.3.5 Multicomponent Alcohol Calibrator Commercial Calibrator [Cerilliant Multicomponent Alcohol Mix Cat # A-057 or equivalent]
e.g., 0.05 g% target concentration.
Storage- Refrigerator Expiration- as labeled

1.3.6 Multicomponent Alcohol Control:
Commercial Control [Cerilliant Multicomponent Alcohol Mix Cat # A-056 or equivalent]
e.g., Target Concentration: 0.10 g%
Storage- Refrigerator Expiration- as labeled

1.3.7 Positive Controls:
Commercial 0.08 g% (Cerilliant or equivalent), Commercial 0.15 g% (Utak or equivalent), and Commercial 0.20 g% (Lipomed or equivalent)
Storage- Refrigerator/Freezer Expiration- see label

1.3.8 Negative Blood:
(Lampire Bovine blood in NaF Cat# 7200815)
Storage- Refrigerator/Freezer
Expiration- 24 months if frozen, 30 days after thawed and then stored in refrigerator.

2 PROCEDURE

Ethanol present in blood samples can be detected and quantified. This method is used to determine ethanol in blood samples. The following compounds can also be determined by this method:

Methanol
Isopropanol
Normal Propanol
Acetone

2.1 Apparatus and Materials

Agilent 8697 Headspace Sampler and Agilent GC 8890.
Chromatography Data System - Agilent OpenLab CDS
Columns: Agilent J&W (DB-ALC1) 30m, 0.320 mm i.d., 1.8 µm film thickness or equivalent (BAC1). Agilent J&W (DB-ALC2), 30m, 0.320 mm i.d., 1.2 µm film thickness or equivalent (BAC2).
20 mL glass headspace vials, butyl stoppers, and aluminum crimp tops.
Manual hand crimper for headspace vials.
Auto-diluter (Hamilton Microlab Diluter)
Internal Standard (0.05% n-Propanol)

Calibration Standards and Controls used:
0.05, 0.10, 0.30, 0.40 g% Ethanol or equivalent
Multicomponent Alcohol (0.05 or 0.10 g%) Cerilliant Standards or equivalent
Commercial positive control (Cerilliant, Lipomed, Utak, or equivalent)
A known target concentration positive control e.g., 0.08 g% ,0.15 g%, 0.20 g% (in blood and/or DI water).
Negative Blood
Additional calibration may be used upon approval from the supervisor.
Deionized Water
Cleaning Wipes
Bleach solution
Syringes – All Teflon with Luer lock fitting for auto-diluter
1.0 mL Hamilton Gas Tight # 81323, Model 1001 TLLX SYR or equivalent

2.2 Evidence Handling

2.2.1 All evidence submissions shall be according to current Lab evidence policies. This policy mandates specific requirements that shall be adhered to, *in addition* to the policies outlined in the *Quality Manual 5.8*. Evidence shall be requested by the analyst using a delivery request in PLIMS or by retrieving the evidence at the Property Control window.

2.2.2 All evidence shall be submitted to the receiving analyst and custody transferred within PLIMS. The receiving analyst shall verify that the outside of the kit is sealed, dated, and initialed. The analyst will mark the evidence, blood kit/package (at a minimum) to show receipt date, analyst initials, and laboratory number. In the event of a discrepancy in the contents or packaging, the analyst will: note the discrepancy in PLIMS, notify the case officer (either directly or by requesting Property Control to notify the officer), and return custody of the item to Property Control for correction. All Blood kits that are not in the process of being tested will be stored in the Chemistry refrigerator.

2.2.3 BAC analysis will be performed on grey top tubes. Blood samples in which the blood cells have been separated from the liquid (serum and/or plasma) are not analyzed (*see 3.5.1* for calculations involving serum and plasma). Analysts should make note of any abnormalities in the packaging of the blood tubes. The name on the blood tube should correspond with the name of the suspect in PLIMS. In instances where the name does not match or correspond with one another, the officer should be contacted to confirm the name as it should appear on the report. If a name discrepancy persists or the officer cannot be contacted, the report may be generated using the following: the name on the blood tube, the name present within/on the kit, or by consulting KBCops. Middle name(s) and/or initial(s) may be removed in PLIMS at the discretion of the analyst to match the evidence and/or for the report. Name determinations made by the analyst shall be noted in the case file and/or PLIMS.

2.2.4 In order to analyze a batch (i.e., one or more samples with controls and calibrators), a sequence will be created in the OpenLab software. A sequence can be created by selecting OpenLab - Acquisition and utilizing sequence templates and/or manual entry to fill out the sequence. See sections 2.3 and 3.2 for sequence guidelines/setup. All selected blood tubes will be run in duplicate; however, blood tubes with a small

volume may be run in singlet. The appropriate calibration standards and controls will be run with every batch.

2.2.5 After the analysis is complete and no repeating of analysis is necessary, the evidence can then be properly resealed and labeled per the "Quality Manual" and returned to Property Control. The transfer will be tracked in PLIMS.

2.3 Initial Setup and Aliquoting

- Create a folder on the Resource drive for the new sequence.
- Record sequence information on the Blood Alcohol Summary Report excel sheet or equivalent. Save to the appropriate folder on the Resource drive.
- Check volume of Internal Standard and settings on the Hamilton Pipettor/Diluter.
- Press the left size button. The display will indicate the size of the syringe on the left side of the instrument. It should read "1000", and the syringe should be a 1.0 mL (1000 µL) Hamilton syringe. If the display is incorrect, press the "increase" or "decrease" arrows until the display indicates "1000".
- Press the right "SIZE" button. The display will indicate the size of the syringe on the right side of the instrument. It should read "1000", and the syringe should be a 1.0 mL (1000 µL) Hamilton syringe. If the display is incorrect, press the "increase" or "decrease" arrows until the display indicates "1000".
- Press the left "VOLUME" button. The display will indicate the volume (in µL) of internal standard solution the pipettor will deliver when the button is pushed. It should read "1000". If the display is incorrect, press the "increase" or "decrease" arrows until the display indicates "1000".
- Press the right "VOLUME" button. The display will indicate the volume of sample (in µL) the pipettor will deliver when the button is pushed. It should read "250". If the display is incorrect, press the "increase" or "decrease" arrows until the display indicates "250".
- Label a series of 20 mL headspace vials to designate the following: Ethanol calibrators, Multicomponent Alcohol Calibrator, Negative Control- #1, Positive Controls- #1, Multicomponent Control, Samples, Negative Control #2, and Positive Controls #2.
- Place blood tubes on the tube rocker or manually invert several times to produce a homogenous sample. Allow all tubes to come to room temperature for approximately 30 minutes. Verify tubes have reached ambient temperature by touch.
- Place tip of pipettor into the calibrator, control, or blood tube/sample container and activate the sample withdrawal by pushing the button. This will pull 250 µL of the sample into the pipettor tip.
- Wipe the tip of the pipettor, place the tip in the appropriately labeled headspace vial, and press the trigger once. This will dispense 250 µL of sample or calibrator into the vial, followed by 1 mL of the internal standard. The internal standard is diluted with sufficient water that it acts as a wash solution, clearing the lines of sample. If sample is still visible, rinse until clear by dispensing deionized water and/or internal standard through the line. Deionized water (or another suitable cleaner and DI water) may also be used to clean the outer tubing if necessary.
- Cap headspace vial with septum stopper/aluminum seal and crimp the seal with the crimper.

- Repeat above steps until all calibrators, controls, and samples have been aliquoted.
- The following is the preferred position allocation of run sequence for case samples:

  Ethanol 50 mg/dL – Level 1 calibrator
  Ethanol 100 mg/dL – Level 2 calibrator
  Ethanol 300 mg/dL – Level 3 calibrator
  Ethanol 400 mg/dL – Level 4 calibrator
  Multicomponent Alcohol mix 500 mg/dL – Level 5 calibrator

  Blank Whole Blood (Neg. Control) 1
  Ethanol 80 mg/dL – Pos. control 1
  Ethanol 150 mg/dL – Pos. control 1
  Ethanol 200 mg/dL – Pos. control 1
  Multicomponent Alcohol mix 1000 mg/dL - Positive control

  Case Samples {up to 20 vials *(batch of 10 blood tubes when run in duplicate)*}.

  Blank Whole Blood (Neg. Control) 2
  Ethanol 80 mg/dL – Pos. control 2
  Ethanol 150 mg/dL – Pos. control 2
  Ethanol 200 mg/dL – Pos. control 2

- Negative and positive controls must be run after every set of case samples (maximum of 20 samples/vials (batch of 10 blood tubes when run in duplicate) per set).

3 GC AND DATA ACQUISITION

3.1 Agilent 8697 Headspace Sampler /Agilent 8890 GC-FID Method

GC Method: *BLOOD_ALCOHOL3.amx*
Data Processing Method: *BA_ANALYSIS3.pmx & BA_VALIDATION3.pmx*
Report Templates: *BA_ANALYSIS.rdl & CalibrationCurveSummary.rdl*
Columns: 30 m x 0.320 mm DB_ALC1 & DB_ALC2 or equivalent
Oven Program: 40ºC (Isothermal)
Detector Temperature: 250ºC
Headspace Oven: 70ºC
Loop Temperature: 80ºC
Transfer Line: 90ºC

Note: Final oven hold may vary to achieve full elution of internal standard

Place all the vials in the tray of the Headspace Analyzer (auto sampler), beginning with position 1. The order should adhere to the guidelines set in section *2.3* and match the information provided on the Blood Alcohol Summary Report.

3.2 Batch Sequencing

- In OpenLab - Acquisition a new sequence can be created or edited. To create a new sequence – click the Create a New Sequence icon followed by New Sequence.
- A sequence list template may be used by selecting “Apply Template”. The number of samples should be entered (this number refers to sample vials only and excludes calibrators, controls, or a double blank) and the appropriate template selected. The Standard BAC Sequence Template should be used for casework and the Validation BAC Sequence Template should be used during appropriate Validation procedures.
- The sequence template should be checked/edited for completeness and correctness (i.e., vial order, sample type, run type, sample name, acquisition method, and processing method) to match the vials on the auto sampler.
- The calibrators should be set to the appropriate Level and Run Type (see *3.3*).
- Save the sequence as a new file using the current date followed by the analyst’s initials, i.e., 070122MJ and/or to match the Sequence ID listed on the Blood Alcohol Summary Report. Subsequently, the sequence will also need to be printed and/or saved as a PDF for inclusion in the PLIMS packet (an equivalent file type that is compatible with PLIMS may also be used).
- Click run.
- After completion of a batch run, verify the identity/location of each vial on the auto sampler against the sequence list prior to removal from the auto sampler.

3.3 Calibration and Data Processing

- The instrument is calibrated with each batch by setting the calibrators at their appropriate calibration curve level via the sequence list or in Data Analysis.
- In OpenLab - Acquisition the sequence list column “Level” can be set to match the appropriate calibration level. Level 1 for 0.05 g%, Level 2 for 0.10 g%, Level 3 for 0.30 g%, Level 4 for 0.40 g%. and Level 5 for Multicomponent Alcohol 0.05 g%.
- Set the sequence list column “Run Type” to the appropriate setting (clear calibration at level, clear all calibration, or leave the field(s) empty/blank).
- Once the run is complete and data is ready to be analyzed, open the OpenLab-Data Analysis program, and load the sequence data into the Data Processing section.
- Under the Data Processing section, link and reprocess the data using the appropriate processing method *(see Appendix A).* The processing method *BA_ANALYSIS*3 will be used for casework and *BA_VALIDATION3* will be used for Validation procedures. The internal standard in each processing method is set to 0.1000 g/100mL for data reporting purposes only (see *1.3.3* for actual concentration).
- Check that the injection data passes all QC requirements and save the results before progressing to the Reporting section of the reprocessed data.

- If the calibration is not set appropriately in OpenLab-Acquisition/the Sequence list, then the calibration Run Type and/or Levels can be corrected post-sequence run via OpenLab-Data Analysis *(see Appendix A).*

3.4 Data Reporting

- In OpenLab - Data Analysis under the Reporting section, select the sequence heading and/or all sequence injections. Click on the report template *BA_ANALYSIS.rdl* and refresh the preview to generate a data packet/report that includes all injections in the sequence. Verify that the data has passed all QC requirements. Print the data packet/report to PDF format and save in the appropriate folder.
- Select the Multicomponent Alcohol 0.05 g% injection data file for the sequence and click the report template *CalibrationCurveSummary.rdl*. Refresh the preview to generate the calibration table and curves report for the sequence. Check that the calibration curves have passed all QC requirements. Print the calibration and curves report to PDF format and save in the appropriate folder.
- Transfer the PDF files of the sequence list, data packet/report, and calibration and curves report to the sequence's folder on the Resource drive.
- Update/complete the Blood Alcohol Summary Report excel sheet to match the data packet/report data. The excel sheet should provide calculation results to help the analyst examine that all QC requirements have been met.
- Attach the contents of the sequence's folder on the Resource drive into PLIMS for each case (i.e., Blood Alcohol Summary Report excel sheet and PDFs transferred from the instrument) for inclusion into the PLIMS packet.
- Create a BAC report in PLIMS for each case, edit the report to display the BAC value, and ready the associated PLIMS packet for review (see section *4.5* and *Appendix B*). Manually generated reports using the BAC report template may be used when necessary.

3.5 Calculations

OpenLab will calculate the concentration of the volatiles and generate a report. Should a manual calculation be required, use the data from the Multicomponent Alcohol Calibrator and the unknown, and insert the appropriate values in the following formula:

$$\text{Concentration in unknown sample} = \frac{(Avp)(Aiss)(Cs)}{(Avs)(Avisp)}$$

Where:

Avp = Area counts of the sample volatile of interest
Aiss = Area counts of the internal standard in the multi volatile calibrator injection
Cs = Concentration of the analyte in the multi volatile calibrator
Avs = Area counts of the multi volatile of interest in the multi volatile calibrator
Avisp = Area counts of the internal standard in the sample injection

Example:
In a hypothetical injection of the multi-volatile calibrator, the area counts for acetone are 252 and the area counts for the internal standard are 451. The concentration of acetone in the Multicomponent Alcohol Calibrator is 0.0500 g%. In our hypothetical sample, the acetone counts are 506 and the internal standard counts are 460. Thus,

Avp = 506 Cs = 0.0500 Avs = 252 Aiss = 451 Avisp = 460

$$\text{Concentration} = \frac{(506)(451)(0.0500)}{(252)(460)} = 0.0984 \text{ g\% Acetone}$$

3.5.1 Serum and Plasma:

Serum is obtained by allowing blood to clot while Plasma is the liquid separated from whole blood with an anticoagulant. For alcohol determination, the concentration in both Serum and Plasma are the same. The average ratio of Serum/Plasma Alcohol concentration to whole blood is approximately 1.18 (Garriot's Medicolegal Aspects of Alcohol *5th edition, page 207*).

Example:
To convert Serum alcohol to whole blood equivalent:
Convert units to g/100mL.
mg/dL = mg/100 mL
mg/100mL = g/100 mL
1000
1204 mg/dL = 0.1204 g/dL (serum alcohol concentration).

$$\text{Whole blood Alcohol concentration} = \frac{\text{Serum/plasma Alcohol concentration}}{1.18}$$

$$= \frac{0.1204}{1.18} = 0.102 \text{ g/100mL} = \text{average whole blood equivalent.}$$

4 QUALITY CONTROL AND REPORTING

4.1 General

4.1.1 Reagents, chemicals, and supplies shall be handled, transported, stored, and used in a manner that preserves their quality. In general, the manufacturer's recommendations for storage conditions as specified on the product label should be followed. Standards and reagents will be verified prior to use in casework.

4.1.2 The precision liquid processor (i.e. Hamilton Microlab Diluter) will be wiped down after each use with a suitable cleaner (i.e. 10% chlorine bleach solution) and deionized water.

4.1.3 All portions of the tubing and syringes will be purged, cleaned, and decontaminated monthly (or as needed) using a cleaner that is compatible with the fluids previously run through the system. Tubing that is in poor condition will be replaced.

4.1.4 The precision liquid processor will be calibrated annually by a certified vendor that is ISO 17025 accredited. The precision liquid processor shall meet the manufacturer's specifications and the mean of the repeatability measurements shall be +/- 1.00% of the expected value. Casework may resume once the certified vendor provides a certificate of calibration and the certificate is reviewed by the Chemistry Section Supervisor to verify all criteria has passed. The calibration certificate will be initialed and dated by the reviewer and retained in the Chemistry Section. An in-house performance verification will be done every year. The calculated percent deviation must be +/- 1.00% to meet acceptable criteria.

4.1.5 New external calibrators will be verified by analyzing and comparing to an existing calibration curve prior to use and/or by direct use in a new calibration curve that provides acceptable control and calibration values. New controls will be verified by analyzing and comparing to an existing calibration curve and meets acceptable control values.

4.1.6 Positive control data will be logged on a Blood Alcohol Control Chart (Levey Jennings plots – located on the R drive).

4.2 Chromatography

Evaluation of the data resulting from the analysis of an unknown sample by GC/Headspace begins with an examination of the chromatogram data followed by a direct comparison of the data with that of a known reference standard.

For identification purposes the analyst will visually examine the GC chromatogram and address (if necessary) any problems with the peaks that are present (i.e., over/under concentration, possible co-elution, peak splitting, or other peak shape problems). All peaks should be symmetrical in appearance, without shoulder or excessive tailing.

There must be baseline separation between analyte(s) and internal standard peaks. The retention times of the analyte(s) peak and the n-Propanol internal standard peak must be within 0.02 minutes of the retention times of the same peaks in the calibrator on the same column.

4.3 Calibration and Controls

4.3.1 If any calibrator fails to meet quality control standards, the curve is invalidated and must be repeated.

4.3.2 The concentrations in the controls must be within 10% of the target. The ethanol concentration in the negative control must be less than 0.0050 g%. Any sample

bracketed in the sequence by a failed control should be reanalyzed. The analyst should evaluate sequences with more than two sets of controls on a case-by-case basis to determine if any failed controls affect all sequence samples or only the bracketed samples and a note shall be made in the case file.

4.3.3 Calibrators must calculate within ±5% or 0.0050 g/dL of the expected calibration value, whichever is greater.

4.3.4 BAC controls will be recorded after each run (Levey Jennings Plots).

4.3.5 The concentrations of the duplicate injections for each case's samples must be ± 5% of the mean of the two injections. If duplicate injections do not meet these criteria, the samples and/or blood tube shall be reanalyzed. If reanalysis of the samples and/or blood tube fails to meet acceptable criteria, then the supervisor will be notified, and the samples will be reported as inconclusive.

4.3.6 The correlation coefficient of the calibration curve must be >0.995.

4.4 Linearity

The linearity of the method is validated on an annual basis. The curve will contain six data points, ranging from 0.01 g% to 0.50 g%. A linear regression analysis (least squares) will be performed on these data. An acceptable correlation coefficient for the range will be 0.995 or greater. The LOD/LOQ will be the lowest standard, which is within ±10% of the target and the ULOL will be the highest standard that falls within ±10% of the target and shows no carryover. The LOD/LOQ and ULOL of the method shall be determined on an annual basis.

4.5 Reporting

4.5.1 The raw data of duplicate injections for a sample shall be averaged. The results shall then be truncated to two digits after the decimal point [e.g., 0.3254 to 0.32 g/100mL] as required by the State of North Carolina (N.C.G.S. §20-4.01). The analyst will manually enter the reported BAC value to the report generated by PLIMS.

4.5.2 Values below 0.02 g% [truncated] should be reported as <0.02g%.

4.5.3 Blood tubes with values above the upper end for method linearity (>0.50 g%) shall be reanalyzed by diluting and re-aliquoting a sample. The sample will be diluted so that the value of the diluted sample falls within the acceptable range (see *4.6 Dilution Table)*. If the calculation of the diluted sample confirms the initial result, then the result will be reported as >0.50g/100mL. If the calculation of the diluted sample is not within ± 5% of the original value, the sample and/or blood tube shall be reanalyzed.

4.5.4 If reanalysis of the sample fails to meet acceptable criteria, then the supervisor will be notified, and the samples will be reported as inconclusive. No uncertainty value will be reported for samples with values >0.50g/100mL.

4.5.5 All final reports must be notarized and copies will be sent to North Carolina Department of Health and Human Services (DHHS) and North Carolina Department of Motor Vehicles (DMV) as required by the state of North Carolina. Final reports will also be sent to the DA's office and the officer associated with the case.

4.6 Dilution Table

Dilution	Amount of Sample units (ml)	Amount of DI Water/Matrix Units (ml)
1:1	1	1
1:3	1	3
1:4	1	4
1:9	1	9

5 PEER REVIEW AND DOCUMENTATION

5.1 Case File Contents

The case file will contain all documentation related to a request for analysis and all communications about the case.

- Administrative Documentation (if available)
- Laboratory Request Forms (if available)
- Laboratory work sheet (if available)
- Case related conversations/correspondence (if any)

The file in PLIMS shall contain, upon completion, a copy of the issued report, any addendum/supplemental reports, and corrected/amended reports.
Each page within the case file should have at a minimum the complaint and/or PLIMS Lab # for the case and the analyst's initials.
The laboratory case files shall be maintained in secured areas of the Crime Laboratory.

5.2 The final inspection of casefiles generated by the chemistry section consists of an administrative and a technical review. The reviews will be documented in the case file with initials and the date of the reviewer in the assigned area of the worksheet or electronically using the case review functions in PLIMS.

5.3 Technical review will be conducted on a minimum of 99% of cases. The technical reviewer shall check for accuracy and clarity by reviewing:

- The examination documentation to ensure the case notes, worksheets, photographs, and other data support the conclusions.
- Manual calculations and data transfers are correct.
- Appropriate procedures and controls are performed and documented.
- General initialing, dating, corrections, and unique case identifiers are correct and appropriate.
- Worksheets are properly utilized and filled out correctly.
- All requested examinations have been performed and addressed on the report or documented by case communication.

5.4 Administrative review will be conducted on 100% of cases to ensure the completeness and correctness of the reports issued. An administrative review will be completed by someone other than the assigned analyst or technical reviewer. The administrative reviewer shall check for accuracy and clarity by reviewing:

- The report for typographical or grammatical errors, misspellings, incorrect dates, omissions, identifying case information errors, or data transfer errors.
- Each page of the examination documentation has the unique case identifier (unless stapled together), initials of the analyst, and no improper corrections.
- Any supporting administrative documentation or case communication contains the unique case identifier (unless stapled together) and the initials of the analyst.
- The case file contains the original lab request or email request, email communications, examination documentation, and a report.

5.5 If a deficiency is detected during the review process, it shall be corrected and, if necessary, a new report generated.

6 UNCERTAINTY OF MEASUREMENT

6.1 General

Estimation of uncertainty shall be determined where the testing contains measurement results that are quantitative, reported, and may reasonably be expected to be used, by an immediate or extended customer (anyone in the judicial process) to determine, prosecute, or defend the type or level of criminal charge(s).

The uncertainty of measurement will include at a minimum the identification and assessment of the major sources of uncertainty in the procedure which are of importance to the process. This may include the methods, instrument/equipment, special environmental conditions, types of evidence tested, the reference standards used, and the operator. The sources of uncertainty will be classified as Type A or Type B components. An uncertainty budget table shall be completed to include both Type A and Type B components of uncertainty for the process of determining blood alcohol concentration. Uncertainty budget should be re-evaluated on an annual basis. The actual uncertainty budget table and calculations/data will be maintained in a separate binder located in the Chemistry Section of the laboratory and/or on the Resource drive.

Calibrators and controls used in the estimation of uncertainty shall be verified to be NIST traceable (*refer to 1.3.1)* by examining the certificate of analysis (CoA). CoAs will be retained in the Chemistry section and/or on the Resource drive. The units of uncertainty values will be measured and/or converted in the same units. All significant figures will be carried throughout calculations used to determine standard uncertainty, combined uncertainty, and expanded uncertainty. Conventional rounding rules will be applied to the expanded uncertainty to determine the reported UOM. The reported UOM shall not exceed two digits after the decimal point as required by the State of North Carolina (N.C.G.S. §20-4.01) and must be a minimum of +/- 0.01 g/100mL. The 99.73% confidence interval will be used for reporting measurement of uncertainty as it pertains to determining blood alcohol concentration.

Identification of Uncertainty Components:

Staff:		Procedure:		Facility:		Calibrators:		Internal Standard:		QC:		Instrumental Analysis:	
knowledge	A	Vortex samples	A	temperature	A	Ref. Material COA	B	purity	B	Ref. Material COA	B	Calibration curve	A
experience	A	Diluting with IS	A	humidity	A	Cal. uncertainty	A	solvent	A	QC uncertainty	A	Integration parameters	A
training	A	Sealing HS vials	A	barometric pressure	A	Volume error	A	Pipetting technique	A	Volume error	A	Injection volume	A
Compliance with SOP	SOP			vibrations	A	pipetting	A	Reading meniscus	A	pipetting	A	Gas flow	A
Multiple staff performing	A			limitations of workspace size	A							Instrument method	A
Operator technique	A											Process algorithm	A
distraction	A												
Mood of operator	A												

6.2 Definitions for Uncertainty of Measurement

Type A evaluation of uncertainty is a method by statistical analysis of a series of observations.

Type B evaluation of uncertainty is a method by means other than the statistical analysis of a series of observations.

The mean is defined as the sum of the measured values divided by the total number of values: $X = \frac{\Sigma x_i}{n}$

Where X is the mean,
xi is the different measured values,
n is the number of measured values

The standard deviation measures how closely the data are clustered about the mean. The standard deviation is defined as

$$s = \sqrt{\Sigma (x_i - X)^2 / n-1}$$

where: s is the standard deviation
X is the mean,
x_i is the different measured values
And n is the number of measured values

The confidence interval is an expression stating that the true mean, μ, is likely to lie within a certain distance of the measured mean, X. The confidence interval of μ is given by:

$$\mu = X \pm \frac{ts}{\sqrt{n}}$$

where s is the measured standard deviation,
n is the number of measured values, and
t is a number from the Student's t table for a certain confidence interval.

Sometimes the values are more likely to fall near the average than further away. This is typical of a *normal* or *Gaussian* distribution. This is graphically represented by a bell curve. Normal distribution uses the above equations for determining measured standard uncertainty.

When the measurements are quite evenly spread between the highest and the lowest values, a *rectangular* distribution is produced. The standard uncertainty for a rectangular distribution is:

$$\frac{a}{\sqrt{3}}$$

Where: a is the semi-range (or half-width) between the upper and lower limits.

6.3 Estimating the Uncertainty of Measurement

The factors to be considered in determining the uncertainty of the measurement are: pipette use, uncertainty associated with preparing the internal standard, preparing quality control samples, preparing blood alcohol samples, and preparing calibrators, repeatability, uncertainty associated with the manufactured standards, instrument variations, and analyst pipetting technique differences.

The Chemistry section relies on the use of quality controls to establish the historical standard deviation for blood alcohol concentration cases.

The quality controls are documented for each batch on the Blood Alcohol Summary Report, Headspace Loadlist, Instrument Worklist, or equivalent.

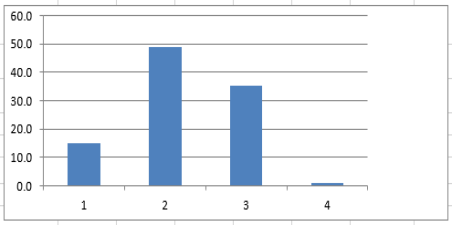
Use of quality controls eliminates most of the uncertainty associated with pipet use, preparation of internal standard, preparation of QC standards, preparation of samples, preparation of calibrators, and analyst pipetting technique differences.

The use of an internal standard for quantitative analysis minimizes other sources of uncertainty including instrument variations. Therefore, this is not included in the uncertainty calculation.

Example of a budget table for Blood Alcohol Analysis:

Measurement Uncertainty Estimation Form

Measurement:	Determining BAC using HS GC-FID (Headspace Sampler 8697/GC 8890)								**Year:** 2024
Range of measurement values:	0.01 g/dL to 0.50 g/dL								
Procedure name and revision:	Blood Alcohol Analysis , 7-22								
Estimation prepared by:	MAJ					**Date Prepared:**	12/21/2023		
Line Item	**Uncertainty Component**	**Value**	**Units**	**Distribution**	**Type**	**Divisor**	**Degrees Freedom (n-1)**	**Standard Uncertainty**	**Component Contribution %**
1	Measurement Process Reproducibility for 0.08	0.00042231	g/dL	normal	A	1.00	87	0.000422308	14.9
2	Measurement Process Reproducibility for 0.20	0.001380158	g/dL	normal	A	1.00	87	0.001380158	48.8
3	Calibrator Uncertainty	0.00200000	g/dL	-	B	2.00	-	0.001000000	35.3
4	Dilutor Uncertainty	0.00005355082	dL	-	B	2.00	-	2.677541E-05	0.9
	Combined Standard Unc u_c							0.001756103	100
	Expanded Unc U (k=2)							0.003512206	
	Expanded Unc U (k=3)							0.005268309	
	Reported Uncertainty:	**0.01**		**k=3**					



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations

The basis for the data above:

1	Measurement Process Reproducibility for the 0.08 Positive Control
2	Measurement Process Reproducibility for the 0.20 Positive Control
3	Accredited Vendor's Uncertainty for the 0.40 g/dL Calibrator concentration at 95% confidence level, k=2 (using the calibrator with the largest uncertainty - converted to proper units)
4	Accredited Vendor's Uncertainty at 95% confidence level, k=2 (converted to proper units from calibration certificate)
Revision:	12/21/2023

Calculation of Combined Uncertainty

The combined uncertainty will be determined when more than one standard uncertainty is shown to be a major contributor to the uncertainty of measurement. The combined uncertainty will be calculated using the Root Sum Squares (RSS) method. The RSS equation is defined as the square root of the sum of the squares of the data points. All significant figures will be carried throughout this calculation.

$$U_{combined} = \sqrt{u_1^2 + u_2^2 + u_3^2}$$

Calculation of Expanded Uncertainty

The expanded uncertainty is used as a measure of uncertainty that defines a confidence interval about the measurement result. It is the combined uncertainty multiplied by the confidence factor (k). All significant figures will be carried throughout this calculation.

$U_{expanded} = U_{combined}(k)$ where k = 3, at the 99.73% confidence level

Using the budget table example above, the expanded uncertainty at the 99.73 % confidence level is:

$U_{expanded} = 0.0018504701$ g/dL (3) = 0.0055514102 g/dL

Using conventional rounding rules, the expanded uncertainty is rounded to two digits after the decimal to produce the reported UOM (minimum value of +/- 0.01 g/100mL):

$U_{reported}$ = +/- 0.01 g/100mL

The uncertainty of measurement for blood alcohol analysis shall be re-evaluated on an annual basis. The technical records, supporting documentation, and calculations used for the uncertainty of measurement evaluation shall be technically reviewed by a second authorized analyst. The reviewer will submit the approved uncertainty of measurement documentation to the Chemistry Section Supervisor for review and final approval. All approvals will be documented and retained in the Chemistry section.

6.4 Recording and Reporting

6.4.1 The measurement of uncertainty will be recorded on the report to the second decimal place to be consistent with the reporting of the alcohol concentration. For example, blood alcohol analysis with an average concentration of 0.1480 g/100mL would be recorded on the Report as 0.14 grams of alcohol per 100 milliliters of whole blood (+/- 0.01 grams per 100 milliliters).

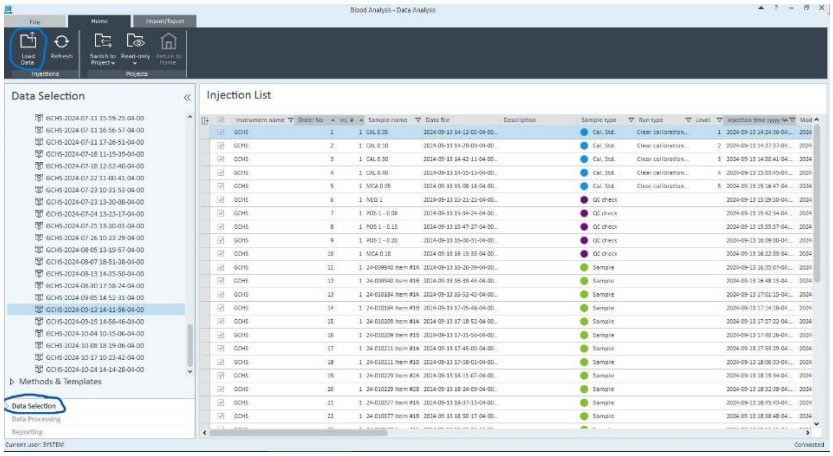
6.4.2 A statement will be added to the report, "All +/- values are reported at a confidence interval of 99.73%."

7 REFERENCES

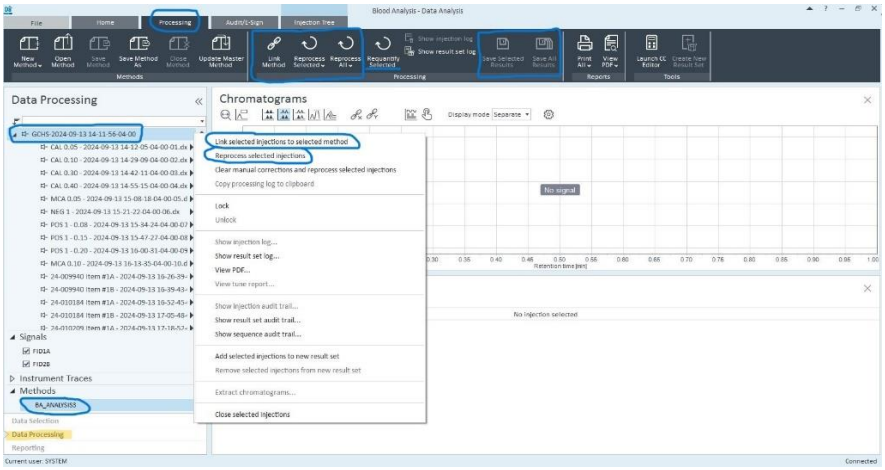
James C. Garriott. Medicolegal Aspects of Alcohol. 5th Ed.
Randall C. Baselt. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed.
B.L. Glendening and R.A. Harvey; "A Simple Method using Headspace Gas Chromatography for Determination of Blood Alcohol by Gas Chromatography." J For Sci 14:136-145 (1969).
Richard Saferstein; Criminalistics- An Introduction to Forensic Science.
R.M. Anthony, D.A. Sutheimer, and I. Sunshine; "Acetaldehyde, Methanol and Ethanol Analysis by Headspace Gas Chromatography." J Anal Tox 4:41-42 (1980).
American National Standards Institute and the American Society for Quality National Accreditation Board (ANAB) manual.
Scientific Working Group for Forensic Toxicology (SWGTOX) Recommendations
ASCLD/LAB-*International* Guidance on Measurement Traceability, May 2013.
ASCLD/LAB-*International* Guidance on Measurement Traceability-Measurement Assurance, May 2013.
ASCLD/LAB-*International* Guidance on the Estimation of Measurement Uncertainty – Overview, May 2013.
NIST/NIJ -The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers, http://dx.doi.org/10.6028/NIST.IR.7928, April 2013
North Carolina General Statue §20-4.01
North Carolina General Statue §20-139.1
Measurement Uncertainty for Weight Determinations in Seized Drug Analysis, Supplemental Document SD-3, SWGDRUG 2011-07-07
ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX B, Drug Chemistry Discipline Three Examples – Weight, Volume and Purity Determination, May 22, 2013, Appendix A to AL-PD-3063 Ver 1.0
Forensic Analysis of Blood Alcohol Concentration, Using the Agilent 8860 GC with Agilent J&W DB-BAC1 UI and Agilent J&W DB-BAC2 UI columns and the Agilent 7697A headspace sampler

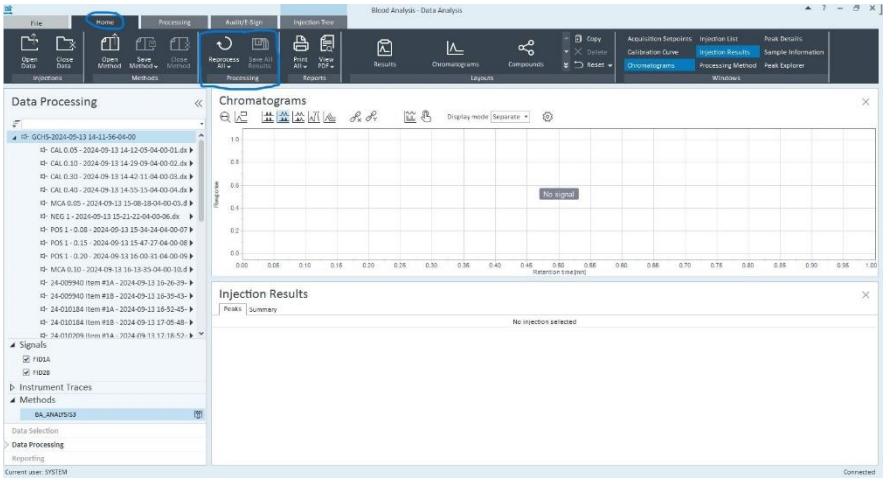
Appendix A:
Processing data for data analysis.

Select/Load sequence data by double clicking the sequence or using the upper pane.

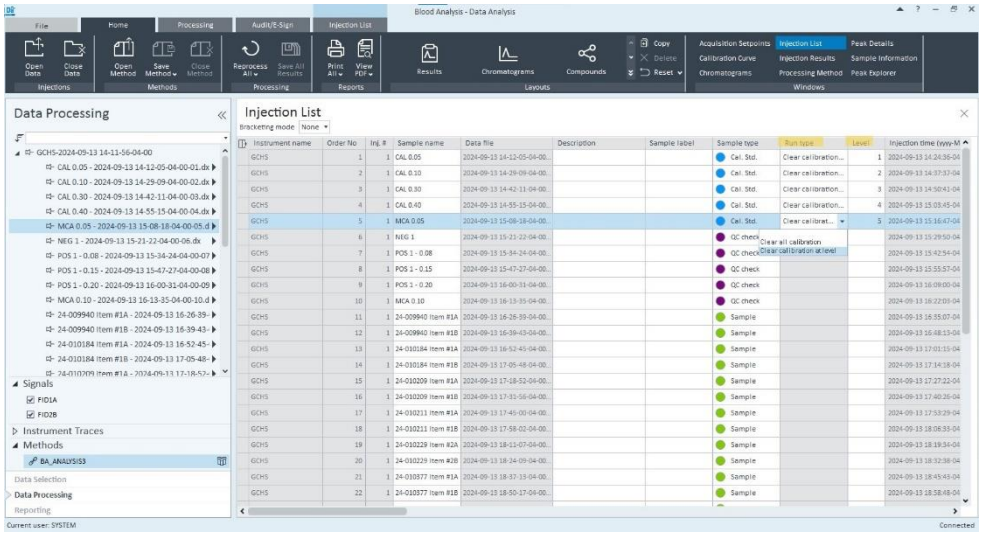


Right click on sequence data heading and/or use upper pane icons to link/reprocess data (using the correct method) and save results.

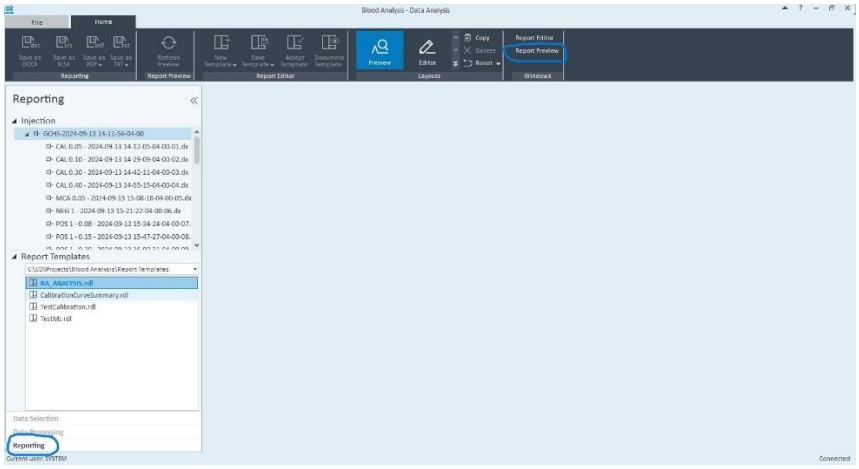




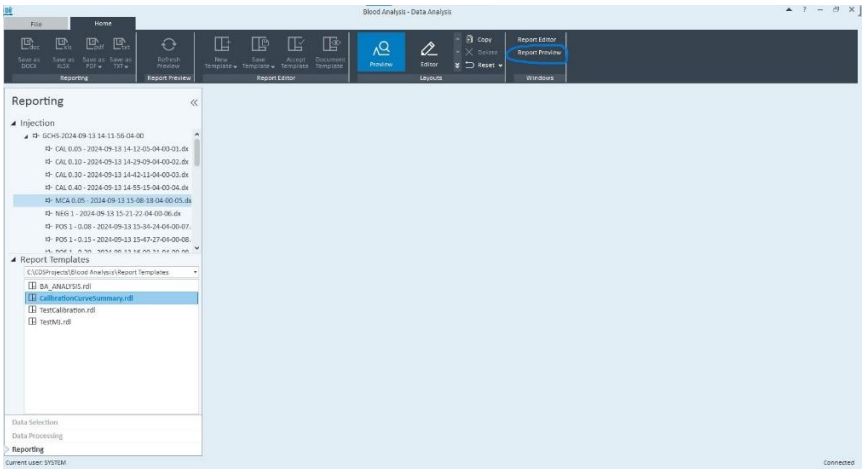
(If Necessary) Correct the Calibration Run Type and/or Levels by editing the Injection List before processing data.



Generate the data packet by selecting the sequence data heading, BA_ANALYSIS.rdl, and then Report Preview. Save/Print as .pdf.



Generate the calibration curve packet by selecting the MCA 0.05 data file, CalibrationCurveSummary.rdl, and then Report Preview. Save/Print as .pdf.



Appendix B:
Instructions for creating a BAC report in PLIMS and setting a BAC packet Ready for Review

Creating a BAC Report:

1. Click the **Analysis** button under the **Assignments** tab.
2. Click the **Report Writing** tab.
3. Click the **Send to Word** button to create the auto-generated BAC report with PLIMS information.
4. Edit the BAC report to the correct BAC concentration value (add Remarks if applicable) and Save.

Setting a BAC Packet Ready for Review:

1. Attach all documents, sequence data, etc. to be included in the notes packet by using the attachment button while on the Assignments tab in PLIMS.
2. Click the Ready for Rev button to bring up a new window of possible packet contents.
3. Examine the documents/contents to be included in the notes packet and click Process.
4. Review the Notes Packet for accuracy and completion. Then click the **Accept** button.
5. Route the BAC packet/report for review.

Jeffrey S. Taylor
Digitally signed by Jeffrey S. Taylor
Date: 2024.12.10 09:16:24 -05'00'