2.1 Determination of Ethanol (Ethyl Alcohol)

2.1.1 General Description of Method

A procedure utilizing gas chromatography-headspace (GCHS-FID) for the determination of ethanol. Two quantitative tests are performed by the addition of an internal standard to an aliquot of the liquid test sample.

2.1.2 Type and Size of Sample

Blood, urine, bile, vitreous humor, other liquid biological sample or alcoholic solution may be used for analysis. A 50 microliter aliquot of sample is used for the quantitative tests. When two blood samples are received on an individual, perform both GC analyses on the one sample closer to the time of the incident. For OWI cases, when two urine samples are received that were voided more than 5 minutes apart, perform both GC analyses on the second or latter urine sample. For all other cases, perform GC analyses on the first or earlier urine sample.

2.1.3 Equipment and Reagents

2.1.3.1 Instrumentation

Thermo Scientific Trace gas chromatographs (Trace GC), each fitted with a flame ionization detector, a CTC Analytics CombiPal or Tri-Plus RSH autosampler, and PC based data system.

2.1.3.2 Required standards:

2.1.3.2.1 Internal Standard Solutions:

- 1 - Propanol, 0.02 gm/dl in deionized water
  (To be used with Rtx-BAC Plus 1 Columns)
- t - Butanol, 0.0078 gm/dl in deionized water
  (To be used with Rtx-BAC Plus 2 Columns)

2.1.3.2.2 Aqueous calibrators purchased from Cerilliant (multicomponent calibrators contain ethanol, methanol, isopropanol, and acetone):

- 0.010 g/dL multicomponent calibrator
- 0.010 g/dL calibrator
- 0.050 g/dL multicomponent calibrator
- 0.100 g/dL multicomponent calibrator
- 0.200 g/dL ethanol calibrator
- 0.400 g/dL multicomponent calibrator

- 0.500 g/dL ethanol calibrator

2.1.3.2.3 Aqueous ethanol controls purchased from Cerilliant:

- 0.020 g/dL ethanol
- 0.080 g/dL ethanol
- 0.150 g/dL ethanol
- 0.300 g/dL ethanol
- 0.400 g/dL ethanol

2.1.3.2.4 Positive Controls

- Aqueous Volatile Mix Control (0.100 g/dL each of methanol, ethanol, isopropanol and acetone)
- Aqueous Low Volatile Mix Control (0.010 g/dL each of methanol, ethanol, isopropanol and acetone)
- Human Whole Blood Ethanol Controls
- 0.005 g/dL Ethanol Control made from the 0.010 calibrator, diluted 1:1 with deionized water

2.1.3.2.5 Negative Controls

- A negative control shall be run following the 0.500 g/dL calibrator, 0.400 g/dL multicomponent control and the volatile mixture (deionized water with internal standard solution)
- A "reverse" negative control shall be run on each column utilized for a casework batch

2.1.3.3 PPE and Miscellaneous

- Disposable gloves, eye protection and lab coats
- Biological safety cabinet
- Automatic pipettor-dilutors
- 20 mL autosampler vials with butyl rubber septa and metal caps
- Crimper for sealing vials
- Sample mixing apparatus
- Assorted support equipment as needed: beakers, flasks, disposable and volumetric pipettes, homogenizing glassware, etc.

2.1.4 Types of Columns

- Rtx-BAC Plus 1 (Restek; 30 m x 0.53 mm ID x 3 µm)
  Recommended for use with 1-Propanol internal standard solution.
- Rtx-BAC Plus 2 (Restek; 30 m x 0.53 mm ID x 1 µm)
  Recommended for use with t-Butanol internal standard solution.

Pertinent information about the columns and GC temperatures will be found on the chromatograms. The oven, injector and detector temperatures used in unknown testing shall be the same as that used for its calibration.

NOTE: *The actual column temperature may vary from column to column as needed to maintain acceptable analysis time and retention time consistency for the analytes.*

NOTE: *At the conclusion of each analytical batch a temperature ramp program should be run to help ensure that any high boiling point contaminants that could damage the column are removed. Do not exceed the column's maximum operating temperature.*

2.1.5 Method

At the beginning of each day, inspect all equipment for proper function and cleanliness, and repair or replace parts when necessary. Verify that sufficient reagents and compressed gases are available for the day's work. Verify that the correct processing method is being utilized on each of the PCs associated with analysis.

Quantitative testing is performed on two Trace GCs, each with a different internal standard. If it is not possible to perform testing on two separate instruments, a single GC may be used. On occasions when this is necessary, two separate aliquots of the sample shall be tested.

GCs are calibrated each day a batch is run, using the concentrations of ethanol, methanol, isopropanol and acetone from the list above to generate calibration curves. When possible, the Cerilliant (NIST traceable) Ethanol Controls, Human Whole Blood Ethanol Controls, the Aqueous Volatile Mix and the Aqueous Low Volatile Mix shall be run following calibration within the batch run as "unknowns" to verify the calibration curve. These control samples will be used to verify and ensure the proper function and accuracy of the instrument, the method, and the calibration curve.

The calibration models and peak detection algorithims for the Trace GC Ultra are as follows:

Analyte	Curve Type	Weighting	Origin	Peak Detection Algorithm
Ethanol	Quadratic	Inverse (1/x)	Ignore	Avalon
Methanol	Linear	Inverse (1/x)	Ignore	Avalon
Acetone	Linear	Inverse (1/x)	Ignore	Avalon
Isopropanol	Linear	Inverse (1/x)	Ignore	Avalon

Calibration models shall not be changed in the processing method or Quan Browser unless validated.

A homogeneous blood sample is assured by gently rocking the specimen on the sample mixing apparatus for at least 5 minutes. If the specimen is clotted, homogenizing glassware can be used to obtain a liquid sample. All sample handling will be performed in the biological safety cabinet using the universal biohazard handling techniques. Only one tube of the submitted sample(s), normally, is tested on each GC instrument. The other, if present, is left in its unopened state.

Each control and unknown biological specimen is processed in the biological safety cabinet by using the two techniques that follow. At the end of each day's testing, all potentially contaminated equipment will be decontaminated with approved solutions provided for that purpose.

2.1.5.1 Biological Specimens

- A 50 µl aliquot of the sample is aspirated by the automatic pipettor-dilutor, dispensed and rinsed with 800 µl of the 1-propanol alcohol internal standard solution into a 20 ml size autosampler vial with butyl rubber septa and metal crimp cap. The vial is placed in its designated position in the autosampler according to the itemized sample list. A headspace sample of this mixture is injected into the appropriate GC.
- A 50 µl aliquot of the sample is aspirated by the automatic pipettor-diluter, dispensed and rinsed with 800 µl of the t-butanol alcohol internal standard solution into a 20 ml size autosampler vial with butyl rubber septa and metal crimp cap. The vial is placed in its designated position in the autosampler according to the itemized sample list. A headspace sample of this mixture is injected into the appropriate GC.

2.1.6 Interpretation of Data

All data interpretation shall be performed in the Quan Browser application of Xcalibur. In order to utilize Quan Browser, the sequence list of samples that were analyzed must be reprocessed. The analyst shall verify that all of the parameters listed in 2.1.5 are correct in the processing method being used. Any parameter not consistent with the information in 2.1.5 shall result in the analyst notifying the unit supervisor of the discrepancy. The retention times of each analyte should be updated if necessary and does not require notification to the unit supervisor. The identity of the detected analyte(s) is established by the agreement of the retention time with previously run standards.

Note: The elution order of analytes in the volatile mixture is:
BAC Plus1: methanol, ethanol, isopropanol, acetone, 1-Propanol I.S.
BAC Plus2: methanol, ethanol, acetone, isopropanol, t-Butanol I.S.

Note: The acceptance criteria for analyte retention time (RT) is ± 0.05 minutes of the RT established in the volatile mixture

Quan Browser provides the analyst with an opportunity to review all analyte integrations prior to printing reports. Processing method parameters are set in a manner that optimizes analyte integrations. However, there may be instances in which particular integrations require manual adjustment by the analyst to ensure the most appropriate representation of the area of the analyte peak is displayed. Cases in which manual integration is performed shall result in the analyst making notation of said integration in the case record comments of the Forensic Advantage LIMS. That notation shall

include the reason the manual integration was necessary, the analyte that was integrated, the date of integration and the initials of the analyst.

After reviewing all integrations and parameters to ensure they are correct, the analyst shall save the Quan Browser file. Alcohol reports may now be printed from the Reports Dialogue menu. Any future data processing that is required shall be performed in the Quan Browser (.XQN) file that was saved.

2.1.6.1 Calibration Curve and Controls

Correlation of determination (r2). The r2 value for the ethanol calibration curve must be ≥0.9990. Acceptable tolerance for ethanol calibrators is ±5% of the target concentration or ±0.005 g/dL, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol calibrators is ±10% of the target concentration or ±0.005 g/dL, whichever is greater. Verification of calibrator lot number and expiration date shall occur at the time calibrator/control packs are reviewed.

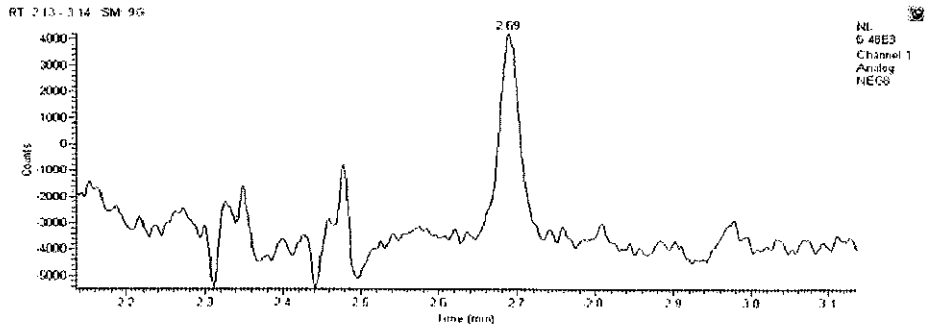
Acceptable tolerance for positive ethanol controls is ±5% of the target concentration or ±0.005 g/dL, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol positive controls is ±10% of the target concentration or ±0.005 g/dL, whichever is greater. Verification of control lot number and expiration date shall occur at the time calibrator/control packs are reviewed.

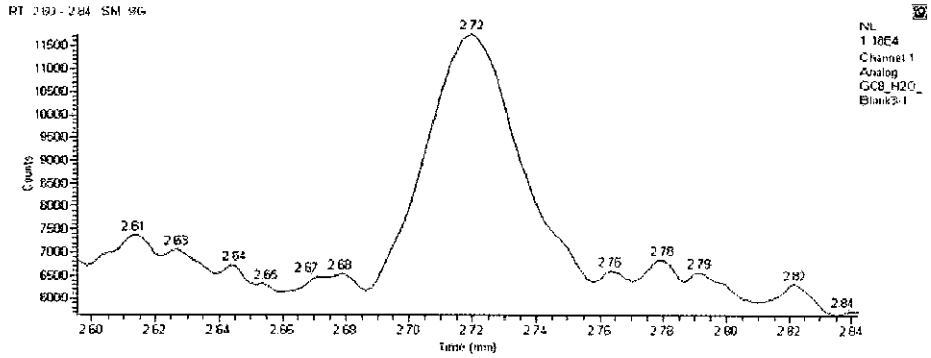
- The target concentration of external whole blood controls shall be established with each new lot. This is accomplished by running the new control 10 times and determining the mean value. The mean must fall within the manufacturer's specified range of the target concentration. The target concentration can be listed as, but is not limited to, "Target Value", "Mean" or "Concentration".
- The target concentration of an internally prepared control is defined as the level at which that control has been prepared.

If one positive control does not meet acceptance criteria on one instrument, no action is necessary. If more than one control does not meet acceptance criteria on one instrument, the analyst will notify the supervisor. The supervisor will determine whether a portion of the run or the entire run will be re-analyzed.

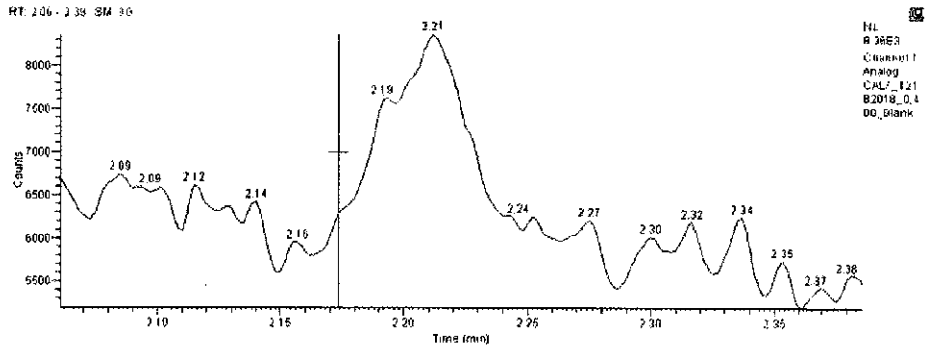
Acceptance of analytical data in any given casework batch requires there to be no ethanol present in negative control samples that is ≥ to the method's LOD (0.005 g/dL). The presence of ethanol in negative control samples that is ≥ 0.005 shall result in immediate consultation with the unit supervisor. Appropriate action shall be taken to eliminate ethanol in negative samples. The "reverse" negative control is used to determine the retention time of the internal standard used on the alternate GC.

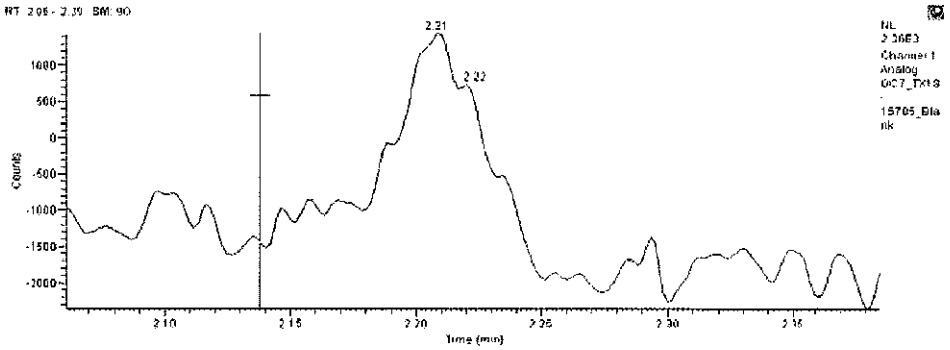
The following are examples of negative control samples within a casework batch that DO require an evaluation of area counts to ensure that they are less than the LOD Ethanol on this Rtx-BAC Plus2 column eluted at 2.69 minutes.





The following are examples of negative controls within a casework batch that DO NOT require an evaluation of area counts to ensure that they are less than the LOD Ethanol on this Rtx-BAC Plus1 column eluted at 2.21 minutes.





These four examples do not encompass every possible scenario that may exist when analysts evaluate negative control samples. Analysts should use the chromatographic quality definitions in protocol 5.7.2 to aid in determining whether negative control samples should/should not be evaluated.

2.1.7 Casework Acceptance Criteria and Reporting Guidelines

The validated limit of quantitation for ethanol is 0.010 g/dL. The validated limit of detected for ethanol is 0.005 g/dL. The linear reportable range for quantitative ethanol results is 0.010 g/dL to 0.500 g/dL.

Reporting guidelines are in compliance with sections 625a, et seq., of Act No. 300.

2.1.7.1 Ethanol in Whole Blood or Vitreous Humor

2.1.7.1.1 Ethanol concentration is ≥0.010 g/dL and ≤0.500 g/dL on both columns:

- The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed. The results from both chromatograms are entered to three decimal places into the Alcohol Worksheet in Forensic Advantage LIMS.
- The resulting average is displayed to three decimal places and shall be reported with the unit of measurement **"grams alcohol per 100 milliliters blood"**.
- The calculated uncertainty statement appears below the results on the final report in the following format: **"The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 100 milliliters blood at the 99.7% level of confidence."**

2.1.7.1.2 Ethanol concentration >0.500 g/dL on one or both columns:

- Consult Supervisor

2.1.7.1.3 Ethanol concentration <0.010 g/dL on one or both columns:

- The following statement shall be typed into the Narrative field: **"Not Detected: Alcohol"**
- No uncertainty statement is required.

2.1.7.2 Ethanol in Urine

2.1.7.2.1 Driving related cases (reported in units: g/67 mL)

2.1.7.2.1.1 Ethanol concentration ≥0.010 g/dL and ≤0.500 g/dL

- The individual positive quantitative urine alcohol results from each chromatogram will be multiplied by a factor of 0.67. The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
- The calculated results will then be entered to 3 decimal places into the in the Alcohol Worksheet in Forensic Advantage LIMS.
- The resulting average is displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 67 milliliters urine".**
- The calculated uncertainty statement appears below the results on the final report in the following format: "**The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 67 milliliters urine at the 99.7% level of confidence.**"

2.1.7.2.1.2 Ethanol concentration >0.500 g/dL on one or both columns:

- Consult Supervisor

2.1.7.2.1.3 Ethanol concentration <0.010 g/dL on one or both columns:

- The following statement is typed into the Narrative field: **"Not Detected: Alcohol"**
- No uncertainty statement is required.

EXAMPLE:

A urine alcohol concentration of 0.249 obtained from one chromatogram is calculated as follows: (0.249 X 0.67 = 0.167). The result of 0.167 is then entered in the Alcohol Worksheet. The same calculation is performed for the second result of 0.255 (0.255 x 0.67 = 0.171) and the calculated result of 0.171 is entered in the Alcohol Worksheet. The urine alcohol result will now be reported as: "0.169 grams alcohol per 67 milliliters urine"

2.1.7.2.2 Non-driving related cases including sexual assault and decedents

- Results **will not** be multiplied by a factor of 0.67.
- Acceptance criteria and reporting guidelines are the same as those found in 2.1.7.2.1.
- The results shall be reported with the unit of measurement **"grams alcohol per 100 milliliters urine".**

2.1.7.3 Serum or Plasma Alcohol

2.1.7.3.1 Ethanol concentration ≥0.010 g/dL and ≤0.500 g/dL

- The results from both chromatograms are entered to 3 decimal places into the Alcohol Worksheet in Forensic Advantage LIMS. This results in obtaining an average concentration and measurement of uncertainty for the serum or plasma that was analyzed.
- Additional calculations must be performed to convert the serum or plasma result to an equivalent whole blood alcohol concentration and to calculate the measurement of uncertainty of the equivalent whole blood concentration.
    - The average result from the Alcohol Worksheet is divided by 1.16 to obtain the equivalent whole blood alcohol concentration.
    - The measurement of uncertainty is calculated by multiplying the equivalent whole blood alcohol concentration by the current expanded uncertainty.
    - Results are entered into the Narrative section of the Alcohol Worksheet, see below example for format.

2.1.7.3.2 Ethanol concentration >0.500 g/dL on one or both columns:

- Consult Supervisor

2.1.7.3.3 Ethanol concentration <0.010 g/dL on one or both columns:

- The following statements are typed into the Narrative field: **"Not Detected: Alcohol"**

No uncertainty statement is required.

EXAMPLE:

Result from chromatogram #1: 0.249 g/dL serum

Result from chromatogram #2: 0.255 g/dL serum

Average of results: 0.252 g/dL serum

The two results agree within ±5% of the average, both results are entered into the Alcohol Worksheet in Forensic Advantage LIMS.

- The results are displayed to 3 decimal places and shall be reported with the unit of measurement **"grams alcohol per 100 milliliters serum".**
- The calculated uncertainty statement appears below the results on the final report in the following format "**The calculated uncertainty of the alcohol measurement is estimated to be ±(concentration) grams alcohol per 100 milliliters serum at the 99.7% level of confidence.**"

The average from above is divided by 1.16 to obtain the equivalent whole blood alcohol concentration:

0.252 / 1.16 = ***0.217***

The uncertainty must be re-calculated by multiplying the equivalent whole blood alcohol concentration by the current expanded uncertainty:

Example of expanded uncertainty : 10.2% = 0.102

Note: use current expanded uncertainty found in protocol 1.11, Measurement Uncertainty, when performing this calculation for casework.

The uncertainty of the equivalent whole blood alcohol concentration is calculated:

0.217 x ***0.102 = 0.022***

The following statements are typed into the Narrative field:

- **"The concentration of this serum (or plasma) alcohol is equivalent to 0.217 grams alcohol per 100 milliliters whole blood".**
- **"The calculated uncertainty of the alcohol measurement is estimated to be equivalent to ±0.022 grams alcohol per 100 milliliters whole blood at the 99.7% level of confidence".**

2.1.7.4 Methanol, Isopropanol and Acetone in Biological Specimens

- The linear reportable range for methanol, isopropanol and acetone is 10 mg/dL to 400 mg/dL (0.010 g/dL to 0.400 g/dL).
- The analyte(s) must be present on both columns in the following concentration(s) for a positive quantitative result to be reported:
    - Methanol ≥ 10 mg/dL (0.010 g/dL)
    - Isopropanol ≥ 10 mg/dL (0.010 g/dL)
    - Acetone ≥ 10 mg/dL (0.010 g/dL)

- The results of the two tests are averaged. The difference between the average and either of the two results should not exceed ±10% or ±5 mg/dL (0.005 g/dL), whichever is greater.
- Both results are entered into the Alcohol Worksheet in Forensic Advantage LIMS as whole numbers (no decimal places).

- The results are displayed to three decimal places, but must be edited to remove the decimal and digits to the right of decimal. If the averaged result is not a whole number, the result shall be rounded up to the nearest whole number.
- The result(s) shall be reported as a whole number(s), (no decimal places) with the units "**milligrams (analyte) per 100 milliliters (matrix).**"
- No uncertainty statement is required.
- Results of <10 mg/dL (0.010 g/dL) on one or both columns are not reported.
- If the methanol, acetone and/or isopropanol concentration is >0.400 g/dL on one or both columns:
    - The sample should be repeated on both columns using a 1:2 (or other appropriate) dilution.
    - The results of the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed.
    - Apply the appropriate dilution factor to the results obtained on each chromatogram to calculate the concentration of the samples.

2.1.7.5 Alcoholic Beverages

Samples identified as suspected alcoholic beverages should be diluted in the manner described below before analysis.

- For beer or beer like beverages, dilute the sample at least in the ratio 1:10.
- For spirits or spirit like beverages, dilute the sample at least in the ratio 1:100.
- Proceed with the analysis as shown for biological specimens in 2.1.5.1. All beverage samples shall have a negative control run following them to rule out any possible carryover.
- Results from the two tests are averaged. The difference between the average and either of the results should not exceed ±5% or ±0.005 g/dL, whichever is greater. If the results fall outside the acceptance criteria, then the sample must be re-analyzed. **(These will not be the results that are entered into the FA worksheet. This step is performed to ensure that the data obtained as a result of analysis meets acceptance criteria.)**
- Apply the appropriate dilution factor to results obtained on each chromatogram.
- Divide the calculated result by 0.79 (specific gravity of ethanol) to convert the result into percent volume/volume.
- The average of the two results is used to determine if the sample has a concentration of greater than or equal to 0.5% by volume.
- The average of the two results is entered into the Alcohol Worksheet in the Beverage Ethanol Result field.
- If the result of analysis is ≥0.5% v/v the following statement is reported:
    - **"Analysis of the sample showed the presence of greater than 0.5% by volume ethyl alcohol and its composition is consistent with an alcoholic beverage".**
- If the result of analysis is <0.5% v/v the following statement is reported:
    - **"Analysis of the sample showed the presence of less than 0.5% by volume ethyl alcohol and its composition is not consistent with an alcoholic beverage".**
- No uncertainty statement is required.

2.1.8 Records

Calibrator and control data shall be collated, reviewed, electronically signed and placed in the **"Controls waiting to be reviewed"** folder. Upon completion of supervisor, or designee, review, calibrator and control data shall be moved into the **"Control Storage"** folder for long term electronic storage.

An Alcohol Batch Worksheet should be completed and maintained with the calibrator and control data.

Each case file shall contain all scientifically valid chromatograms specifically associated with that case.

2.1.9 Bibliography

There are several good review articles on alcohol testing that show the general scientific acceptability of the method detailed here.

Jones AW: Measuring Alcohol in Blood and Breath for Forensic Purposes - A Historical Review; Forensic Sci Rev 8: 13; 1996

Tagliaro F, Lubli G, Ghielmi S, Franchi D, Marigo M: Chromatographic Methods for Blood Alcohol Determination; J Chromatography 580:161; 1992

Goldberger, B, Caplan YH, Shaw, RF: Methods for Fluid Analysis, in Garriott's Medicolegal Aspects of Alcohol, 5th Ed., Lawyers and Judges Publishing Co, 2008, p. 255 - 268.

Jones, G and Liddicoat, L: Quality Assurance, in Garriott's Medicolegal Aspects of Alcohol, 5th Ed., Lawyers and Judges Publishing Co, 5th ed., 2008, p. 269 - 274.

5.1 Controls

5.1.1 Blood Alcohol Controls

The following 8 controls are tracked:

- 0.020 g/dL ethanol control
- 0.080 g/dL ethanol control
- 0.150 g/dL ethanol control
- 0.300 g/dL ethanol control
- 0.400 g/dL ethanol control
- Volatile Mixture
- whole blood ethanol level 1 control
- whole blood ethanol level 2 control

An electronic file of all results is maintained and available for evaluation of data trends. The data will be evaluated a minimum of once per calendar year when the uncertainty budget is evaluated.

5.1.2 Toxicology Controls

All toxicology controls run are maintained in an electronic file for evaluation of data trends. The data will be evaluated a minimum of once per calendar year when the uncertainty budget is evaluated.

An extraction worksheet will be filled out by the analyst for every batch of samples extracted. This worksheet will detail lot numbers of chemicals used, as well as the date and initials of the analyst who prepared reagents used in the extraction. This extraction worksheet will be maintained with the toxicology controls for each batch extracted.