Wilmington Police Department
Crime Laboratory

Forensic Alcohol Analysis

Standard Operating Procedure

Table of Contents

1.0 Introduction

Forensic alcohol analysis (FAA) involves analyzing blood, other biological specimen and/or aqueous solutions for the presence and concentration of ethanol (alcohol). The types of cases analyzed include Driving While Impaired (DWI) cases and cases involving a question of alcohol involvement or content. This procedure applies to all forensic samples analyzed by the crime laboratory for the presence and concentration of ethanol.

1.1 Goals

To provide timely forensic alcohol results
To provide scientifically sound expert testimony on the accuracy and reliability of forensic alcohol testing
To provide expert testimony when interpreting alcohol levels and impairment as it relates to driving
To function as a resource on alcohol analysis and impairment to the department and the community

1.2 Objectives

To provide forensic alcohol results within forty-eight (48) hours of a rush request and within ten (10) business days of the date the laboratory receives the request for analysis
To provide training on alcohol analysis and interpretation to members of the law enforcement community, the District Attorney's Office and any other interested parties

1.3 Authorization

The method approved to perform forensic alcohol analysis in the laboratory is permitted by the Department of Health and Human Services (DHHS) – Forensic Tests for Alcohol (FTA) Branch and is an accepted method of testing in the forensic science community. Each individual analyst performing the tests holds a valid permit from DHHS.

2.0 Operations

2.1 Duties, Responsibilities, Accountabilities and Qualifications

Laboratory personnel performing these procedures are qualified and authorized according to the Quality Management System and are responsible to adhere to all policies and procedures therein.

2.2 Security

Laboratory personnel adhere to security measures detailed in the Quality Manual, Section 2.3

2.3 Safety

Laboratory personnel maintain the highest level of safety while working in the laboratory. Refer to MSDS catalogs and the Chemical Hygiene Plan prior to use of any chemicals and other laboratory equipment.

A list of important phone numbers is posted in the laboratory for quick access to emergency and key personnel.

2.4 Evidence Control

Refer to Quality Manual, Section 2.8 and the Evidence Control SOP, QP102.8 for evidence control procedures.

3.0 Training

3.1 Introduction

This training module is designed and intended to prepare a Forensic Chemist or eligible Forensic Lab Technician to be fully qualified to perform Forensic Alcohol Analysis for the Wilmington Police Department Crime Laboratory. The training manual gives the trainee instructions and activities to complete, including references to read and questions to answer. A qualified analyst acts as a mentor to oversee the trainee's activities in the assigned module(s). The training mentor initials and dates the trainee's activity checklist in each module as the activities are completed.

The Forensic Alcohol training program is modular. The modules can be completed in any order, at the discretion and guidance of the training mentor. Each module includes the amount of time estimated for completion. After completion of each module, the trainee and the training mentor will date and initial the training summary. The trainee can perform the duties covered in each module once the module is completed and the trainee is authorized. A certification of training completion is issued by the training mentor at the completion of Modules 3.1 – 3.6.

3.2 Orientation

The Forensic Chemist is assigned to two primary analytical sections of the Wilmington Police Department Crime Laboratory: the Forensic Alcohol section and the Forensic Drug Chemistry section. Both sections activities are directed and coordinated by the Forensic Lab Manager who is responsible for the efficient operation of the laboratory.

Forensic Chemists are trained to function in both sections and called on to work in either section at a given time. Generally, the training is completed in the forensic blood alcohol section first followed by training in the forensic drug section.

The forensic alcohol section primarily receives samples of blood but may receive other biological specimens or beverages for determination of the alcohol concentration. The forensic alcohol section is responsible for all activities related to this function, including receiving samples, analysis, reporting and expert testimony.

All work in the section is performed in compliance with the Wilmington Police Department Crime Laboratory Quality Manual, Quality and Technical Procedures, the Property and Evidence Section Manual, the Wilmington Police Department Policy Manual, the City of Wilmington Policies and Directives and all applicable statutes of the North Carolina General Statutes.

This module should be completed in one week.

3.2.1 Activities

a. Read Forensic Alcohol Analysis SOP, 1.0 Introduction and 2.0 Operations.

3.3 Receiving

3.3.1 Introduction

Receiving is the process of accepting samples into the laboratory for analysis. Completion of this section of the training manual qualifies the new forensic scientist to perform this function.

This module should be completed in one week.

3.3.2 Activities

a. Read Forensic Alcohol Analysis SOP, 6.0 Sample Management, subsections 6.1, 6.1.2, 6.1.3 and 8.0 Procedure, subsection 8.1.1

b. Observe the training mentor perform the process of receiving of samples.

c. Receive samples under the supervision of the training mentor.

d. Answer the questions below:

1. Laboratory report number labels should be placed on which items of the kit?

2. How do you confirm that the blood tubes submitted in a kit were assigned the proper case number?

3. If the blood draw time written on the blood tube is different than the time written on the inner kit box or the consent form:

    a. Which time do you report?

    b. What else do you do?

    c. Is this a major or minor discrepancy? Why?

4. What procedure should be followed where a blood kit is received empty?

5. What procedure should be followed if a blood kit is received in which the inner kit information does not match the outer kit information or the paperwork?

    a. Is this a major or minor discrepancy? Why?

3.4 Blood Analysis Training

3.4.1 Introduction

This portion of the training manual prepares the new forensic chemist to perform the most important function in the forensic alcohol section: Headspace Gas Chromatography.

While a new forensic chemist may complete all the training, casework may only be performed by a forensic chemist qualified as a Chemical Analyst holding a Permit to Perform Chemical Analyses of Blood issued by the North Carolina Department of Health and Human Services under the authority of G.S. 20-139.1 (b).

During training the new forensic chemist will be assigned a training mentor. The training mentor reviews all written answers for correctness and discusses any incorrect answers with the trainee. The training mentor determines if incorrect answers require additional reading or training in the topic. The training mentor reviews all practical exercises performed by the trainee to determine if competency is met.

After completing the first two subsections of this module the forensic chemist may then complete the third subsection which will prepare the forensic chemist to testify about the accuracy and reliability of the blood alcohol results.

Sections 3.4.2 – 3.4.5 of this module should be completed within 6 weeks.

Section 3.4.6 of this module should be completed within 2 weeks.

3.4.2 Sample Preparation

In forensic alcohol analysis, it is important that the procedure is followed consistently and certain criteria are met in order to assure the accuracy and reliability of test results.

3.4.2.1 Activities

a. Read Forensic Alcohol Analysis SOP, 6.0 Sample Management, subsection section 6.1.1 and 8.0 Procedure

b. Read Forensic Alcohol Analysis SOP section 13.2.1

c. Perform the procedure listed in Forensic Alcohol Analysis SOP section 13.2.1

d. Describe the difference between accuracy and precision

3.4.3 Headspace Gas Chromatography

Headspace Gas Chromatography is the procedure used for the routine analysis of casework samples of blood, urine, and liquids suspected of containing alcohol.

3.4.2.2 Activities

a. Read Garriott Chapters 1, 5, 9, 10, and 11

b. Read the Forensic Alcohol Analysis SOP sections 4 – 9

c. Observe the mentor (or other qualified analyst) do one run of casework samples for GC analysis.

d. Perform the procedure in section 13.1.1.1 on both the Trace GC Ultra and Trace GC 1310. Complete an Individual Volatile Retention Time Determination form, TF201.6, for each different column and submit with supporting documentation.

e. Perform an accuracy and precision run on the Trace GC Ultra (see 13.1.1.2).

f. Perform an accuracy and precision run on both columns in the Trace GC 1310 (see 13.1.1.2).

g. On the Trace GC Ultra, conduct two (2) different valid runs of analysis using methods and procedures from the Forensic Alcohol Analysis SOP Manual. Each run shall contain ten (10) replicates of a previously quantitated secondary alcohol standard and three replicates of a Cerilliant standard.

h On the Trace GC 1310, conduct two (2) different valid runs, one on each column if available, of analysis using methods and procedures from the Forensic Alcohol Analysis SOP Manual. Each run shall contain ten (10) replicates of a previously quantitated secondary alcohol standard and three replicates of a Cerilliant standard. Use the same secondary alcohol standard and Cerilliant so results can be compared.

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i. Competency Test (practical): Conduct two valid GC analysis runs. Each run shall contain one replicate of 30 previously analyzed casework blood samples. The mean of the trainee's analyses must agree within 0.010 % (w/v) of the qualified analysts' results. If this criteria is not met the trainee should consult the training mentor and complete 3.4.2.2 sections c-f again.

j. Review the operator manuals for the current instrument software, gas chromatographs, and headspace autosamplers.

k. Read the references (as available) and answer the questions below.

3.4.4 References:

3.4.4.1 Gas Chromatography:

A Full Evaporation Headspace Technique with Capillary GC and ITD, Schuberth et.al., 34 Journal of Chromatographic Science 314-319 (1996).

Chromatographic Methods for Blood Alcohol Determination, Tagliaro et.al., 580 Journal of Chromatography 161-190 (1992).

Determination of Ethanol in Biological Samples by Head-Space Gas Chromatography, Molina et.al., 10 Journal of Pharmaceutical & Biomedical Analysis 1069-1071 (1992).

Evolution of Capillary Columns for Gas Chromatography, Ettre et.al., 19 Liquid Chromatography Gas Chromatography North America 48-59 (2001). (optional)

Flame Ionization Detectors, Hinshaw et.al., 8 Liquid Chromatography Gas Chromatography 104-111.

Method for Determination of Ethyl Alcohol for Medicolegal Purposes, Kozelka et.al., 13 Industrial and Engineering Chemistry 905-907 (1941).

Rapid Vapor Phase Method for Determining Ethanol in Blood and Urine by Gas Chromatography, Wallace et.al., 46 The American Journal of Clinical Pathology 152-154 (1966).

The Beginnings of Headspace Analysis, Ettre et.al., 20 Liquid Chromatography Gas Chromatography North America 1120-1129 (2002).

3.4.4.2 Salting Out:

Salting-Out Effect of Sodium Fluoride and Its Influence on the Analysis of Ethanol by Headspace Gas Chromatography, Jones et.al., 18 Journal of Analytical Toxicology 292-293 (1994).

3.4.4.3 Blood Source and Coagulation:

Blood Alcohol Analysis: Comparison of whole Blood Analysis by Gas Chromatography with Serum Analysis by Enzymatic Method, Shajani et.al., 22 Canadian Society of Forensic Science 317-320 (1989).

Blood Source and Alcohol Level; Errors from Using Venouse Blood During Active Absorption, Harger et.al., Chemical Testing Programme 212-218 (1962).

Comparison of Ethanol Concentrations in Blood, Serum and Blood Cells for Forensic Application, Charbelois et.al., 0 Journal of Analytical Toxicology (0).

Comparison of Plasma, Serum, and Whole Blood Ethanol Concentrations, Winek et.al., 11 Journal of Analytical Toxicology 267-277 (1987).

Distribution of Ethanol Between Plasma and Erythrocytes in Whole Blood, Payne et.al., 217 Nature 963-964 (1968).

3.4.4.4 Preservation

Alcohol Loss Arising from Microbial Contamination of Drivers' Blood Specimens, Dick et.al., 34 Forensic Science International 17-27 (1987).

Effect of Different Concentrations of Sodium Fluoride on Blood Alcohol Determination by Headspace as Chromatography Using the Internal Standard Method, Solanky et.al., 18 Journal of Analytical Toxicology 63 (1994).

Ethanol Oxidation by Erythrocytes, Smalldon et.al., 245 Nature 266-267 (1973).

Inhibition of Ethanol Production by Saccharomyces cerevisiae in Human Blood by Sodium Fluoride, Amick et.al., 42 Journal of Forensic Science 690-692 (1997).

Intragastrointestinal Alcohol Fermentation Syndrome, Kaji et.al., 24 Journal of Forensic Science Society 461-471 (1984).

Possible Sources of Ethanol Ante- and Post-Mortem, Corry et.al., 44 Journal of Applied Bacteriology Jan-56 (1978).

Storage of Specimens at 4 deg Celsius or Addition of Sodium Fluoride (1%) Prevents Formation of Ethanol in Urine Inoculated with Candida albicans, Jones et.al., 23 Journal of Analytical Toxicology 333-336 (1999).

The Bacterial Production of Ethyl Alcohol, Blackmore et.al., 8 Journal of Forensic Science 73-78 (1963).

The Effect of Microbial Contamination of the Blood Sample on the Determination of Ethanol Levels in Serum, Blume et.al., 60 American Journal of Clinical Pathology 700-702 (1973).

3.4.4.5 Stability

Improved Recovery and Stability of Ethanol in Automated Headspace Analysis, Christmore et.al., 29 Journal of Forensic Sciences 1034-1044 (1984).

The Stability of Ethanol in Human Whole Blood Controls; An Interlaboratory Evaluation, Dubowski et.al., 21 Journal of Analytical Toxicology 486-491 (1997).

The Stability of Ethanol in Stored Blood, Brown et.al., 66 Analytica Chimica Acta 271-283 (1973).

The Stability of Ethanol in Stored Blood, Smalldon et.al., 66 Analytica Chimica Acta 285-290 (1973).

The Stability of Ethanol in Stored Forensic Blood Samples, Shajani et.al., 22 Canadian Society of Forensic Science 335-339 (1989).

The Stability of Ethyl Alcohol in Forensic Blood Specimens, Chang et.al., 8 Journal of Analytical Toxicology 66-67 (1984).

The Stability of Ordinary Blood Alcohol Samples Held Various Periods of Time Under Different Conditions, Glendening et.al., 10 Journal of Forensic Science 192- 200 (1965).

3.4.4.6 Other:

Endogenous Ethanol 'Auto-Brewery Syndrome' as a Drunk-Driving Defense, Logan et.al., 40 Medicine, Science, and the Law 206-215 (2000).

Effect of using alcoholic and non-alcoholic skin cleansing swabs when sampling blood for alcohol estimation using gas chromatography, McIV et.al., 44 British Journal of Clinical Practice 235-236 (1990).

3.4.5 Questions

1. Define mobile phase, stationary phase, retention time, relative retention time, resolution, and sensitivity.

2. How is the calibration curve generated? What is the area ratio?

3. What is the difference between internal standard method and external standard? What are the advantages and disadvantages of these methods?

4. What's the purpose of He gas, H_2 gas and air used in our GC instruments?

5. Why is the method employed by our lab called headspace gas chromatography? Describe how a sample is introduced onto a column.

6. Define polarity of a chemical. Is ethanol polar?

7. Describe how a mixture of volatile substances can be separated into individual compounds in our GC columns.

8. Describe the principle behind the flame ionization detector (FID) for ethanol.

9. Is the GC/FID method specific for ethanol? Why or why not?

10. What criteria would you use in selecting a GC column for ethanol analysis?

11. What criteria would you use in selecting an internal standard for forensic alcohol analysis?

12. Is it necessary to know the concentration of N-propanol internal standard in our method? Why or why not?

13. What are the volumes of sample, standard and internal standard pipetted by a diluter in our method?

14. Our method currently uses secondary alcohol standards close to 0.01, 0.05, 0.10, 0.25 and 0.50 in concentration. Why do we select these levels?

15. How is the value of a secondary alcohol standard determined? How is it different from a QC's value determination?

16. Can the expected value of the QC change after it has been determined? Why or why not?

17. Do the calibrators need to be in any particular order or particular position in a run? Do the QC's, calibration check samples and water blanks need to be in a particular position?

18. Why is the second water blank right after the standard of high alcohol concentration?

19. What is PCS an abbreviation of?

20. What's the purpose of a PCS mix in a run?

21. In our method what is the acceptable range of a QC? Of a secondary standard result?

22. Should analysts check chromatograms after a run? Why or Why not? What should be evaluated?

23. What's the detectable level of ethanol in our columns?

24. When is a third analysis required? When is a fourth analysis required?

25. Calculate the % w/v of an unknown using the following information from the chromatograms:

Samples	Sample Peak Area	Internal Std Peak Area
0.199 STD	349831	873861
Unknown	188041	877955

26. How can an outlier be treated in the calculation of experimental results? Calculate the average % w/v of ethanol at 98% confidence using the following results: 0.259, 0.280, 0.283, and 0.279.

27. Secondary alcohol standards are prepared in aqueous solution while unknown samples are in blood. Does a different matrix affect the accuracy and precision of results?

3.4.6 Completion

Upon completion of the activities above, the analyst may apply to the North Carolina Department of Health and Human Services for qualification as a Chemical Analyst.

3.5 Reporting

3.5.1 Introduction

Reporting describes the process of technical review of results, Sample Information Log entry, analyst verification, and administrative review of reports prior to release. The forensic alcohol analyst needs to be familiar with the steps involved in each of these processes.

This module should be completed within one week.

3.5.2 Activities

a. Read Garriott Chapter 12

b. Read Forensic Alcohol Analysis SOP sections 9 – 11

c. Observe qualified analyst(s) perform the steps listed below.

Technical review of a blood alcohol run
Verification and analyst signature
Entry of a Results into Sample Information Log
Preparing a written report
Technical and administrative peer review
Notarizing reports
Distributing reports

d. Prepare a written report

e. Complete the WPCL FAA Uncertainty of Measurement Training

f. Review course material from "Introduction to Measurement Uncertainty in Forensic Chemistry and Toxicology." RTI International (Online) or enroll in online course if available.

g. Competency Test (written): Complete the comprehensive written examination provided by the training mentor.

3.6 Court Testimony (Analysis)

3.6.1 Introduction

The Forensic Alcohol section provides expert testimony on the theory and operation of blood alcohol analysis and the accuracy and reliability of blood alcohol results. The forensic chemist must complete Section 4.2 of the Court Testimony Training SOP, QP102.9.1 in order to testify to the accuracy and reliability of the blood alcohol analysis and results.

3.7 Re-training

3.7.1 Introduction

In the event that a qualified analyst is hired from an external laboratory, a previously qualified analyst has not performed testing in the Forensic Alcohol discipline for more than one year and has not maintained proficiency, or a current analyst fails a proficiency examination; the analyst must participate in a modified training program to ensure competence and proficiency in the area of Forensic Alcohol Analysis.

3.7.2 Orientation (Re-orientation)

The analyst must read the Forensic Alcohol Analysis Standard Operating Procedure, TP101, in its entirety. The analyst must thoroughly review the initial training program for

new Forensic Chemists. The following questions and activities must be correctly completed (a modified Training Sign-off Sheet may be used):

a. 3.3.2 (d)

b. 3.4.2 (c)

c. 3.4.2.2 (c-f)

d. 3.4.5

e. 3.4.6 (If necessary)

f. 3.5.2 (c-d)

g. 3.6.2 (b)

After completion of retraining, the analyst must be authorized to begin analysis on casework. The analyst must pass an external proficiency examination within one (1) year of the completion of retraining to maintain authorization.

4.0 Instrumentation, Equipment and Supplies

4.1 Instrumentation and Equipment

4.1.1 Gas Chromatograph with Flame Ionization Detector, Headspace Auto Sampler, Data System and Restek BAC-1 Column 30m x 0.32mm ID, 1µm or Restek BAC-1 Column 30m x 0.53mm ID, 3µm and Restek BAC-2 Column 30m x 0.53mm ID, 2µm (two column system) or equivalent BAC column(s)
4.1.2 Auto Diluter, Dual Syringe (optional Remote Actuated Footswitch)
4.1.3 Electronic Rocker/Mixer, Speci-Mix
4.1.4 Analytical Balance with Internal Calibration
4.1.5 Hotplate Stirrer
4.1.6 Electronic Crimper and Decapping Pliers
4.1.7 Refrigerator/Freezer (Frigidaire)
4.1.8 Digital thermometers
4.1.9 NIST traceable thermometer
4.1.10 Adjustable pipettes (1 – 5 mL, 100 – 1000 µL, 20 – 200 µL, 5 – 50 µL, 0.5 – 10 µL)
4.1.11 Sonicator

4.2 Supplies

4.2.1 Glass Headspace Vials, 20 mL capacity, aluminum crimping cap with seal
4.2.3 Glass Vials, ½ dram capacity, screw caps
4.2.4 Disposable sampling cups, 1 oz capacity
4.2.5 Disposable plastic transfer pipets, 3 mL capacity
4.2.6 Disposable glass transfer pipets with rubber bulbs

4.3 Performance Checks and Calibrations

Refer to Quality Manual, Section 2.5 and the Maintenance SOP, TP103. Records of performance checks and calibrations are maintained according to Quality Manual, Section 1.13 and the Quality and Technical Records SOP, QP101.13.

4.4 Maintenance

Refer to Quality Manual, Section 2.5 and the Maintenance SOP, TP103. Records of preventive and routine maintenance are maintained according to Quality Manual, Section 1.13 and the Quality and Technical Records SOP, QP101.13.

5.0 Reagents, Standards and Controls

Refer to the Quality Manual, Section 2.1 and the Reagent Check SOP, QP102.1 for reagent check and labeling requirements.

5.1 Reagents and Reference Standards

5.1.1 Absolute Ethanol, Ethyl Alcohol, 200 Proof (USP Grade)
5.1.2 Methanol, isopropanol, acetone, n-propanol, acetaldehyde (Reagent grade or higher)
5.1.3 Reference standard ethanol solutions [e.g. National Institute of Standards and Technology (NIST) or NIST traceable]
5.1.4 Reference standard ethanol, methanol, acetone, isopropanol (e.g. Cerilliant Multicomponent Alcohol Mix or equivalent)

5.2 Standards

5.2.1 Internal Standard (IS): n-propanol
The internal standard is used for the quantitative determination of ethanol. Presence of consistent peak areas and retention times of the internal standard in each sample ensure reliability of the accuracy and precision of the determined ethanol value.

5.2.1.1 Internal Standard (n-propanol) Preparation

Target Concentration: 0.05% n-propanol (0.05 grams n-propanol/100 mL water)

From a bottle of n-propanol, weigh out approximately 0.050 gram for every 100 mL of solution. Decant in an Erlenmeyer flask. Bring flask to volume, mix using magnetic stir bar and magnetic stirrer. Place flask at the diluter station and set up for dilution of samples, standards and controls with the diluter. Label the flask with reagent identification, lot number, date prepared, preparer's initials, date placed into service and date to be discarded (14 days from date of preparation). A pre-printed label may be made available for this purpose. Maintain a log of all internal standards that are prepared and discarded on the Internal Standard Preparation Log, TF201.9. Completed log forms are maintained in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.2.2 Calibration Standards
The calibration standards are used to calibrate the forensic alcohol method by the process of linear regression along a five point calibration curve ignoring the origin (0,0). They are analyzed at the beginning of every run to calibrate each run. Prepare the solutions using absolute ethanol (USP grade) diluted with ultra-pure water. Solutions expire one (1) year from date of preparation.

5.2.2.1 Calibrator Solutions Preparation

Target Concentration: 0.50% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.5 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

Target Concentration: 0.25% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.25 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

Target Concentration: 0.10% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.10 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

Target Concentration: 0.05% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.05 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

Target Concentration: 0.01% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.01 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.2.3 Linearity Check Standards

The linearity check standards are used to check the linearity of the calibration curve at the end of the run. This is to confirm that the linearity of the curve has remained intact throughout the analysis. Prepare the solutions using absolute ethanol (USP grade) diluted with ultra-pure water. Solutions expire one (1) year from date of preparation.

5.2.3.1 Solution Preparation: Linearity Check Standards

Target Concentration: 0.08% Ethanol linearity check solution

From a bottle of absolute ethanol, weigh out approximately 0.08 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

Target Concentration: 0.36% Ethanol calibrator solution

From a bottle of absolute ethanol, weigh out approximately 0.36 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Alcohol Standard Preparation Log, TF201.7. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.2.4 Performance Check Standard

The performance check standard is used to show complete separation of known analytes from ethanol. This standard is run at the end of every run. Prepare the solution as a mixture of volatiles using absolute ethanol (USP grade), methanol, acetone, acetaldehyde and isopropanol (each reagent grade or higher) diluted with ultra-pure water. Solution expires one (1) year from date of preparation.

5.2.4.1 Solution Preparation: Performance Check Standard

Target Concentration: 0.10% Each of ethanol, methanol, acetone, acetaldehyde and isopropanol

Weigh out approximately 0.10 gram of each volatile for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the Performance Check Standard Preparation Log, TF201.16. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.2.5 Individual Volatiles Standards

Standard solutions of each volatile used in the performance check standard are prepared to establish expected elution order and retention time of the individual volatile. Prepare each solution in separate volumetric flasks using methanol, acetone, acetaldehyde and isopropanol (each reagent grade or higher) diluted with ultra-pure water. Solutions expire one (1) year from date of preparation.

5.2.5.1 Solution Preparation: Methanol, Acetone, Acetaldehyde, Isopropanol Standards

Target Concentration: 0.10% Each of methanol, acetone, acetaldehyde and isopropanol

Prepare 100 mL of each solution in separate volumetric flasks. Weigh out approximately 0.10 gram of each volatile separately for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the Individual Volatile Standards Preparation Log, TF201.15. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.3 Controls

5.3.1 Quality Control Standard

The quality control standard is used to check the entire system from sampling to analysis. This standard is run in the middle of forensic samples within the run. Prepare the solution using absolute ethanol (USP grade) diluted with ultra-pure water. Discard one (1) year from the date of preparation.

5.3.1.1 Solution Preparation: Quality Control Standard

Target Concentration: 0.10 – 0.20% Ethanol quality control solution
From a bottle of absolute ethanol, weigh out approximately 0.10 – 0.20 gram for every 100 mL of solution. Decant into the appropriate volumetric flask. Bring flask to volume, mix and dispense into an Amber bottle. Record information on the appropriate Quality Control Standard Preparation Log, TF201.11. Logs are stored in the Alcohol Standards binder located in the laboratory until they are archived electronically.

5.3.2 Negative Control

The negative control is used to show the absence of analyte and carryover in the column. This standard is run before the calibration standards and after the highest concentration calibrator. Ultra-pure water is used for this control.

5.4 Working Solutions Labeling and Storage

Use Amber bottles to store standards and control solutions. Label according to Quality Manual, Section 2.1. Refer to the MSDS for storage requirements for all standards and controls. Use the primary source MSDS to determine storage requirements for secondary standards and controls.

5.5 Sample Preparation

Transfer each of the calibrator, control and linearity check standards into separately marked or labeled ½ dram vials. Samples of each are drawn from these vials to be diluted and analyzed as required by the headspace gas chromatography method. Empty and refill vials on each new day of analysis.

5.6 Establishing Elution Order and Expected Retention Times of the Volatiles

Analyze each of the volatiles that are found in the performance check standard individually followed by the performance check standard. Identify the retention time of each volatile from the individual runs and locate them on the chromatogram of the performance check standard to establish elution order. Record the retention times from the individual volatiles and the elution order from the performance check standard on the Individual Volatile Retention Time Determination form, TF201.6. Accepted retention time range is ±2% of the known retention time to the same significance that the instrument records the retention time. Store forms in the Alcohol Standards binder located in the laboratory until they are archived electronically. A technical review is performed for each determination. Elution order and retention times are established when new standards are prepared, the column length is altered, or operational parameters are changed.

5.7 Determining the Values of the Standards

Follow the Headspace Gas Chromatography Method and Procedure as found in Sections 7.0 and 8.0 of this SOP.

5.7.1 Calibration Standards and Linearity Check Standards

Use NIST traceable standard ethanol solutions (e.g. Cerilliant) to create, at minimum, a five-point calibration curve. NIST traceable standards are used for the linearity check standards until their values are determined. Analyze each standard in replicates of ten (10) on two (2) separate days. Find the mean value and standard deviation of each standard. Record results for each standard on a separate Alcohol Standard Determination form, TF201.8, and store in the Alcohol Standards binder in the laboratory until they are archived electronically.

The relative standard deviation (%RSD) must be less than 5%. Refer to 11.0 Calculations for how to calculate the %RSD. If the %RSD is greater than 5% after the first 20 replicates, additional replicates are analyzed in batches of 10. For example, if the %RSD is greater than 5% analyze 10 more replicates (for a total of 30 replicates) and recalculate the %RSD using all 30 replicates. Continue this process until the %RSD is met or consult the Quality Manager for further guidance.

5.7.2 Quality Control Standard

Using the prepared calibration and linearity check standards, once values are known, analyze the quality control standard(s) twice per day for ten (10) days to determine the mean value and standard deviation. Use a NIST traceable standard for a quality control standard in all runs until the value of the prepared quality control standard is determined. Record results on the Quality Control Standard Determination form, TF201.12, and store in the Alcohol Standards binder in the laboratory until they are archived electronically.

The %RSD must be less than 5%. Refer to 11.0 Calculations for how to calculate the %RSD. If the %RSD is greater than 5% after the first 20 replicates then additional

replicates of the quality control standard(s) are analyzed twice per day until until the %RSD is met or until a total of 30 replicates have been analyzed. If the %RSD is still not met, consult the Quality Manager for further guidance.

5.8 Safety

Wear personal protective equipment (PPE) such as lab coats, gloves and eye protection when working with these solutions. Refer to the MSDS for each reagent prior to initial use and for more information about handling, safety and storage.

6.0 Sample Management

6.1 Headspace Sample Management Procedure

In order to track samples and assure proper identification, analyst(s) follow a series of steps throughout the receiving, sampling, GC sequencing and reporting process. This process is to maintain record keeping, sample entry and result reporting and to avoid and identify errors throughout the process.

6.1.1 Sample Preparation

All forensic samples, reagents, standards and controls equilibrate to room temperature prior to sampling. Blood samples are placed on an electric rocker/mixer for at least two - five minutes prior to sampling to ensure sampling of a homogenous mixture. All other samples are at room temperature and swirled or rocked prior to sampling.

6.1.2 Assigning a Laboratory Report Number (LR#)

All forensic alcohol samples are assigned a Laboratory Report number (LR#) according to Evidence Control SOP, QP102.8. Proficiency samples are assigned laboratory report numbers exactly as case samples and reports are issued.

6.1.3 Entering Sample in Database

After a LR# is assigned to a sample, information about the sample is entered into the Sample Information Log according to Quality System Manual, QD001 and Evidence Control SOP, QP102.8.

6.1.4 Creating a GC Run Sequence

Using the instrument software, create a list of calibrators, controls and samples to be included in the run. Run size is limited to a maximum of twenty (25) forensic samples. Identify calibrators with the notation "CalibX" (X being the number calibrator that it represents) followed by the known concentration, as the Sample ID in the sequence. For example, if the first calibrator has a concentration of 0.011%, then it is identified as Calib1 0.011. Identify the negative control(s) with "H_2O blank". Identify other standards such as the Quality Control (QC) standards and the Linearity Check (LC) standards with their initials and known concentrations (e.g. QC 0.100). External QC samples are identified with the initial E-XXX and the known concentration followed by the Lot number. For example, a Cerilliant sample that should be 0.200 %W/V with a known values of 0.199 %W/V and a lot number of FN01132014-01 is identified on the run sequence as (E-200 0.199 FN01132014-01).
Identify all unknown samples using the Laboratory Report number (LR#) assigned to that sample.

All GC run sequences are set up as follows:

Position in Sequence	Calibrator, Blank or Standard
1	Negative control (H_2O blank)
2-6	Calibrators 1-5
7	Negative control (H_2O blank)
Halfway between case samples	Quality control standard
After case samples and before linearity check samples	External quality control standard (x2) e.g. Cerilliant
After all forensic (unknown) samples	Linearity check standards (two)
Last position	Performance check standard (PCS)

6.1.5 Saving a GC Run Sequence

Save the GC Run Sequence in the folder with the date of the run in the "Data" folder for that instrument. The file name for a GC Run Sequence follows the format of analyst's initials, date sequence created (the date the sampling took place) and the letter A, B, C, etc. corresponding to the number of runs for that analyst that day (i.e. A = 1st run, B = 2nd run, etc.) For example, if analyst BPP is saving the first run sequence on July 1, 2009, the file name is BPP07012009A.

6.1.6 Running a Sequence on the GC

Once a sequence is created and saved, the analyst checks the sequence to ensure that all the information in the sequence is correct and matches the order of the vials in the auto sampler tray before starting the run.

6.1.7 Creating and Retrieving a Run Summary

6.1.7.1 Checking Chromatograms
Once the run is complete the chromatograms are viewed using the instrument software. Peaks in the chromatograms must demonstrate good chromatographic performance through peak resolution (>1.5) and peak shape and should be located within the expected range for retention time. Peaks in the performance check standard are checked for proper elution order.

6.1.7.2 Checking the Calibration Curve and Fit Analysis (R^2 value)
The R^2 value is checked to confirm it is greater than 0.995 or 99.5%.

6.1.7.3 Reviewing a Run Summary
A run summary is automatically generated at the end of the run. This summary is automatically saved in the folder for the date of the run. A run summary is reviewed by the analyst before the run is accepted, pertinent data is transferred to a run summary template which is printed for the technical review and report processing.

The following criteria are required for a run summary to be accepted as valid and the results to be used for reporting:

Standards whose values are < 0.100% (W/V) must have results within ± 0.005% (W/V) of the known value
Standards whose values are ≥ 0.100% (W/V) must have results within ± 5% of the known value.
The quality control standard value must be within ± 0.010% (W/V) of the known value.
The negative control must have a value of less than 0.005% (W/V)
The %RSD of the internal standard areas must be <10%.

6.1.8 Technical Review of the Run Summary

Technical review of a run summary is performed by someone trained and authorized to perform the review other than the analyst who diluted and analyzed the run. Required criteria for the technical review are in Section 10.0, Quality Control.

6.2 Sampling

Prior to sampling cases samples, the analyst confirms that the subject name and LR# assigned to the evidence matches that listed in the Sample Information Log. A print out of this information is checked, initialed and dated by the analyst verifying the review and is filed in the monthly run summary sheet file for the applicable month that the run and review is conducted.

All calibrators, controls and unknown samples are sampled, diluted and analyzed in the exact order in which they appear in the run sequence. Every forensic sample is sampled and analyzed twice on two separate runs. Additional sampling and analysis are required when two sample results do not meet the criteria located in Section 10.0, Quality Control.

6.3 Sample Disposal

All disposable sample cups and GC vials with blood/biological fluids are disposed of in a double-bagged biohazard container. Individual containers of blood and body fluids that are less than 20 mL each are disposed of in regular waste containers inside of biohazard bags.

6.4 Safety

Wear personal protective equipment (PPE) such as lab coats, gloves, and eye protection when working with biological samples. N-95 respirators are optional but available and recommended for use during the sampling process.

7.0 Method

7.1 Headspace Gas Chromatography Method

7.1.1 Summary

An aliquot of blood, biological specimen or aqueous solution is sampled, diluted with an internal standard (IS) solution (n-propanol) into a glass headspace vial, sealed and placed in the incubation oven of the automatic sampler and heated. The concentration of ethanol in the diluted sample is directly proportional to its concentration in the headspace. A portion of the headspace vapor above the liquid is automatically injected into the gas chromatograph (GC) with a flame ionization detector (FID). Ethanol, methanol, isopropanol, acetone, acetaldehyde and n-propanol are identified by retention time and the ethanol concentration is calculated by comparison to peak areas of the calibration standards and the internal standard. Acceptable validated methods must be followed for all instrumentation used to determine ethanol content. The use of headspace gas chromatography is a widely accepted method for forensic alcohol determination.

7.1.2 Scope and Limitations

Specificity – This method is specific for ethanol. There are no known compounds in the blood of living humans that are known to interfere with this method.

Sensitivity – This method is limited to determining the concentration of ethanol solutions that fall between values of the lowest calibrator to the highest calibrator. Any values that fall below the lowest calibrator are reported as "0.00 %" and those that fall above the highest calibrator will be reported as greater than (>) the value of the highest calibrator.

Linearity – The calibration curve is linear and the concentration of ethanol is determined by first-order linear regression when the residual square value (R^2) is greater than 0.995 or 99.5 %.

Limit of Quantitation – The limit of quantitation for this method is the lowest calibration standard.

The reporting limit for this method is defined to be 0.01% (W/V).

8.0 Procedure

8.1 Headspace Gas Chromatography Procedure

8.1.1 Receiving

All evidence analyzed by this method is received from the Property and Evidence Section. Each item of evidence is assigned and labeled with a Laboratory Report number (LR#) and the item number if there is more than one item. A pre-printed label may be made available for this purpose. Once the samples are signed into the custody of the chemical analyst, the analyst will record all pertinent information into the Sample Information Log.
Note: Two blood tubes submitted in one evidence DWI kit are identified as one item.

8.1.2 Sampling

Prior to sampling cases samples, the analyst confirms that the subject name and LR# assigned to the evidence matches that listed in the Sample Information Log. A print out of this information is checked, initialed and dated by the analyst verifying the review and is filed in the monthly run summary sheet file for the applicable month that the run and review is conducted.

All calibrators, controls and checks are sampled directly from ½ dram vials. A small volume of blood, biological specimen or other liquid is transferred by pouring into a disposable plastic sampling cup to be sampled (this process ensures the visualization of clotting and prevents contamination of the original sample container). Initial and date the sample vial to indicate that a sample has been withdrawn from the vial by whom and when.

8.2 Diluter Set-up Procedure

The Hamilton Microlab 530B Dual Syringe diluter is programmed with a sampling program, used to aspirate 200 µL of sample, then to dispense the sample, together with 1800 µL of internal standard, into a glass headspace vial.

The Main Menu of the controller allows the user to choose one of the following options (See the Hamilton Microlab 500B/C User's Manual for programming options):

Run an existing method
Create a new method
Edit an existing method
Manual dilution
Manual dispense
Prime the fluid path
Utilities menu

8.2.1 Prime the System

Select "Prime the fluid path." The system has been set to complete 20 cycles at syringe prime speed 2. Press the "Run/Stop" key to start the priming cycle. Check to make sure the fluid path is free of air bubbles. The instrument displays the status of the priming cycle, *e.g.* "Priming…3 of 20 strokes completed." Upon completion of the priming cycle, the instrument will display "Priming…20 of 20 strokes completed. Prime complete. Press any key to continue." The diluter is now ready for sample preparation. Always prime the diluter before diluting forensic samples.

8.2.2 Dilute the Samples

Select "Run an existing method." Choose method "BAC1." The BAC1 program executes the following sequence of steps:

Step #	Auto	Dly	Volume (Left Syringe)	Speed	Volume (Right Syringe)	Speed
1	Y		FIL 2500 µL	4		
2	Y		DIS		ASP	
3	N				ASP 200 µL	2
4	N		DIS 1800 µL	2	DIS 200 µL	2
5	N		DIS 700 µL	2		

This program is utilized for all forensic samples, calibrators, controls and checks diluted for forensic alcohol analysis by headspace gas chromatography.

Place the diluter delivery tip into the ½ dram vial or the specimen and ensure that the tip is below the surface of the sample. Activate the diluter using the handheld pipet or foot pedal to draw 200 µL of the sample from the vial or sampling cup. Withdraw the tip and wipe with a Kimwipe. Insert the delivery tip into the headspace vial labeled for that sample and activate the diluter to dispense the 200 µL of sample and 1800 µL of the internal standard into the vial. Flush the diluter into a waste beaker. Wipe the tip with a Kimwipe.

Seal all headspace vials by crimping aluminum caps on the vials. Load the sampling tray with the vials in the same order that they were diluted.

8.3 Departures from Methods and Procedures

The method and procedure in this SOP are to be followed for all forensic alcohol analyses. If any departures from these methods and procedures are determined to be necessary, they must be reviewed and approved by the Forensic Lab Manager per the Quality System Manual, Section 1.1.5.

8.4 Discovery Procedures

All documentation is discoverable for a legal proceeding. Requests for documentation made through the proper discovery outlet are fulfilled by making documentation available to the requesting party by providing copies to them or by scheduling an appointment to review on-site. The Forensic Lab Manager determines the appropriate avenue to provide the requested information.

9.0 Parameters

9.1 Headspace Analysis

9.1.1 Gas Chromatography Operational Parameters

The following parameters are recommended as starting parameters. Parameters may be adjusted to maximize performance. Current parameters are posted within the view of the instrument operator.

Trace Ultra Gas Chromatograph	
GC Oven temperature:	40 °C
Hold time:	2 min
Inlet temperature:	200 °C
Split/Splitless mode:	split
Split ratio:	30:1
Column pressure:	24.5 psi
FID base temperature:	250 °C
FID H2 pressure:	30 psi
FID air pressure:	300 psi
FID make-up gas:	He
FID He pressure:	20 psi
Column: Rtx-BAC1, 30m x 0.32mmID, 1µm	

Trace 1310 Gas Chromatograph	
GC Oven temperature:	40 °C
Hold time: 2 min (Front); 3 min (Back)	
Inlet temperature:	200 °C
Split/Splitless mode:	split
Split flow:	214 mL/min
Column pressure:	10 psi
FID base temperature:	250 °C
FID H2 pressure:	35 mL/min
FID air pressure:	350 mL/min
FID make-up gas:	He
FID He pressure:	20 mL/min
Columns: Rtx-BAC1, 30m x 0.53mmID, 3µm; Rtx-BAC2, 30m x 0.53mmID, 2µm	

9.1.2 Headspace Sampler Operational Parameters

The following parameters are recommended as starting parameters. Parameters may be adjusted to maximize performance. Current parameters are posted within the view of the instrument operator.

TriPlus Headspace Autosampler	
Incubator temperature:	80 °C
Incubation time:	15 min
Incubation mode:	Normal
Syringe temperature:	85 °C
Enable pre-filling:	Yes
Extractions:	1
Filling volume:	1 mL

TriPlus RSH Headspace Autosampler	
Incubator temperature:	80 °C
Incubation time:	15 min
Incubation mode:	Constant
Syringe temperature:	85 °C
Enable pre-filling:	Yes
Filling counts:	1
Filling volume:	1 mL

9.1.3 Retention Time Parameters

The expected retention times for methanol, ethanol, isopropanol, acetone, acetaldehyde and n-propanol are determined using the posted parameters for the respective instrument. These retention times are recorded in the Alcohol Standards binder and are re-evaluated whenever new standards are prepared, the column length is altered, or operational parameters are changed.

10.0 Quality Control

10.1 Standards and Controls

10.1.1 Internal Standard (IS): n-propanol

The internal standard is used for the quantitative determination of ethanol. The internal standard is used to dilute all standards, controls and samples to be analyzed in a run. A peak for the internal standard must be present in the chromatograms of each item analyzed in run. Each internal standard peak must have a retention time within ± 2% of the expected retention time and all %RSD of the internal standard areas must be less than 10% for the run to be considered valid and the results of the run to be used for reporting.

10.1.2 Calibration and Linearity Check Standards

The calibration standards are analyzed as the second – sixth samples on every run. They are placed in order from the lowest to the highest known value. The linearity check standards are placed at the end of the run after the last unknown sample. They are run from lowest to highest known value and are analyzed immediately before the performance check standard. Values for the calibration standards and linearity check standards must meet the following criteria in order for a run to be accepted as valid and the results to be used for reporting:

Standards whose values are < 0.100% (W/V) must have results within ± 0.005% (W/V) of the known value. Standards whose values are ≥ 0.100% (W/V) must have results within ± 5% of the known value.

10.1.3 Performance Check Standard

The performance check standard is analyzed as the last sample on every run. The retention time for each volatile in the performance check standard must be present in the chromatogram of the performance check standard in the proper elution order and must demonstrate good chromatographic performance through peak resolution (>1.5) and peak shape and should be located within ± 2% the known retention time for a run to be accepted as valid and the results to be used for reporting.

10.1.4 Quality Control Standard

The internal quality control (QC) standard is placed halfway between all unknown samples in a run. The quality control standard value must be within ± 0.010% (W/V) of the known value. The value of the quality control standard is recorded in the Quality Control Log, TF201.13, each time it is analyzed. The log is stored in the Alcohol Standards binder in the laboratory until it is archived electronically.

10.1.5 Negative Control

The negative control is used to show the absence of analyte and carryover in the column. This standard is always analyzed as the first sample in the run and after the highest

concentration calibrator. Ultra-pure water is used for this control. The value of the negative control must be "Not Found" or < 0.005% (W/V).

10.1.6 External Quality Control Standards

A NIST or NIST-traceable standard (e.g. Cerilliant) with a value between the highest and lowest calibration standards is analyzed twice on each run where unknown samples are being analyzed. Results of the two analyses are recorded in the External Quality Control Log, TF201.14. The log is stored in the Alcohol Standards binder in the laboratory until it is archived electronically. Completed pages of the log are stored electronically per the Quality and Technical Records SOP, QP101.13.

10.1.7 Control Charts

Control charts are updated by the analyst as data points are obtained. . The control charts are reviewed on a quarterly basis by the Quality Manager.

10.2 Non-conforming Work

When a standard or control is analyzed and the above required criteria are not met, the entire run fails and the results are not accepted. The analyst re-samples and re-analyzes the entire run. If the same standards or controls fail again, then the analyst stops work and reports the nonconformance to the Quality Manager. The nonconformance is addressed using the Nonconforming Work SOP, QP101.9.

10.3 Proficiency Testing

Proficiency testing is required at least once per year per analyst to show that the method and procedure for forensic alcohol analysis are producing valid and accurate results. Proficiency tests meet the criteria in Quality System Manual, QD001, Section 2.9. Records of all proficiency testing are maintained per the Quality and Technical Records SOP, QP101.13.

10.4 Refrigerators and Freezers

The temperature of refrigerators and freezers which store reagents, standards or evidentiary material are checked and recorded on a weekly basis using the Refrigerator and Freezer Temperature Log, TF201.17. Logs are located at each refrigerator/freezer that is monitored. Completed logs sheets are scanned and stored electronically. For refrigerators, the temperature should be between 2 – 8 ° C. For freezers, the temperature should be below -20 ° C.

If temperatures fall outside the range, the thermostat should be adjusted. If necessary, the contents of the refrigerator or freezer should be moved to another refrigerator or freezer. Critical reagents and standards should be re-verified if the temperature in the refrigerator exceeds 15 ° C or the freezer exceeds 0 ° C prior to use in case work.

10.5 Measurement Uncertainty

Measurement uncertainty is estimated for forensic alcohol analysis using the simplified Guideline to Uncertainty Measurement (GUM) eight step approach. These steps are

outlined in the following paragraphs. The measurement uncertainty estimate is reviewed on an annual basis when new calibration standards are put into use or more often as deemed necessary by the Quality Manager. The Measurement Uncertainty Form, TF202.10.5, is used to document each measurement uncertainty estimate performed. Any calculations or data used for the estimate not documented on the form are included as attachments.

The coverage probability used for measurement uncertainty estimates is 99.8%. The measurement uncertainty estimate is reported to three decimal places and the results of any calculations will be rounded up, in order to maintain a conservative estimate. During estimation ensure all units are the same for calculations, data can be converted to percentage to simplify calculations.

10.5.1 Simplified GUM Approach

First step is to define what is being measured, which can be done with a short statement or a quantitative expression. Figure 10.5.5 gives an example of the Measurement Uncertainty Form, TF202.10.5, completed with all eight steps.

The second step is to identify potential sources of uncertainty that contribute to the final result's uncertainty. This can be done in a list format or a graphical format. An example of a graphical format for forensic alcohol analysis is shown in Figure 10.5.1.

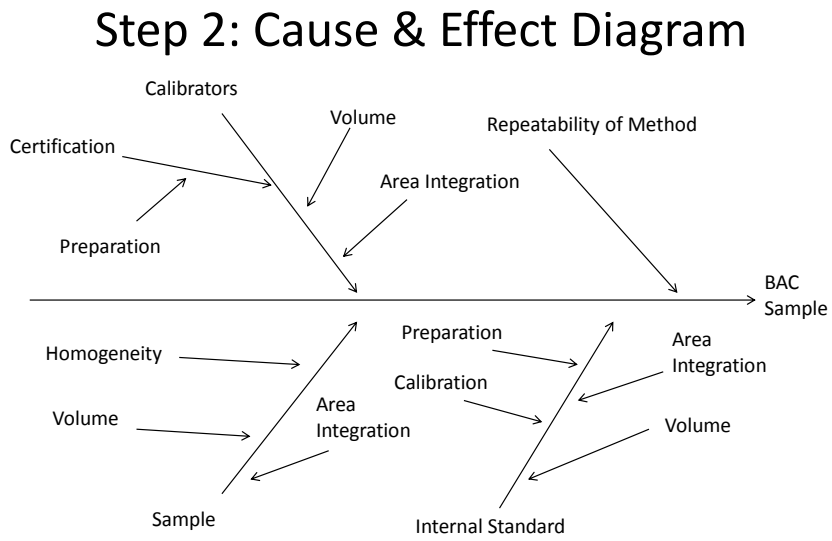

Figure 10.5.1 Potential Sources of Uncertainty

The third step is to reconcile these sources of uncertainty to see if any are adequately accounted for with existing data and can therefore be removed from consideration. Figure 10.5.2 shows how these data can be reconciled for forensic alcohol analysis.

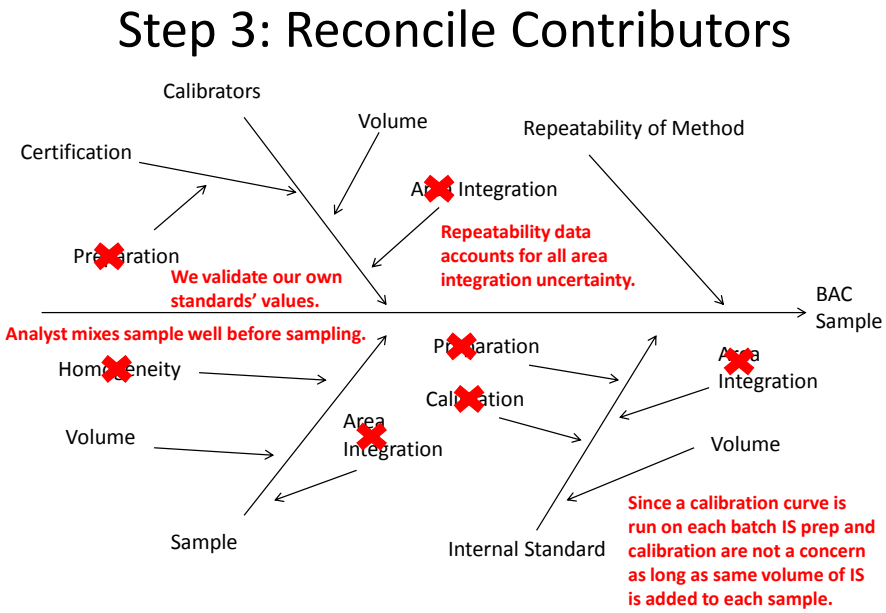

Figure 10.5.2 Reconcile Sources of Uncertainty

The fourth step is to quantify the sources of uncertainty. Figure 10.5.3 shows the remaining sources of uncertainty that affect the final uncertainty of the result. The analyst performing the estimate determines if data already exists for all sources or if further research or experimentation is required.

Step 4: Quantify Uncertainty Sources

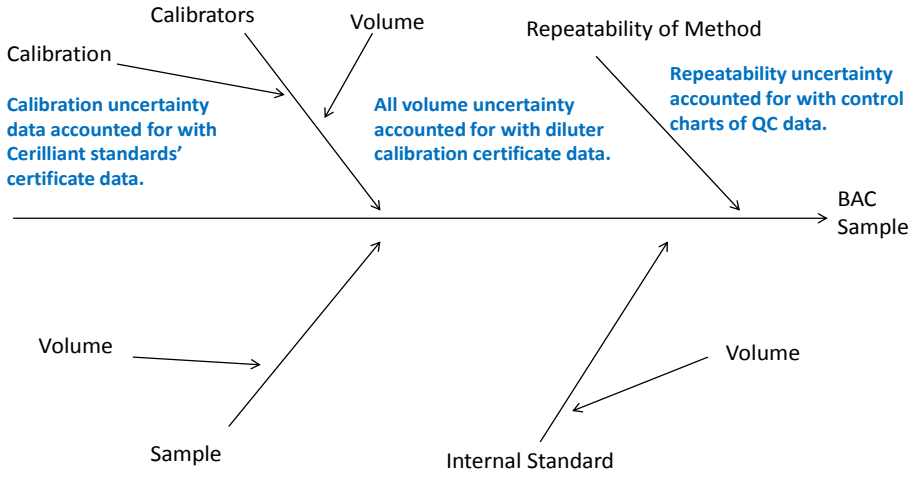

Figure 10.5.3 Remaining Sources of Uncertainty

In addition to determining a value for the uncertainties the characteristics of the data used must be identified as well. The data is categorized as Type A or Type B error and the distribution model is identified.

Type A data is derived from multiple measurements conducted over a period of time and statistically analyzed, this data usually comes from control charts of QC data or validation studies. If data does not exist for a new or unique method then experimentation is performed to obtain reproducibility data. Type B data is not measured statistically by the lab, is usually considered individually, and can be minimized through optimization; type B data usually consists of instrument or standards' calibration performed by an outside source.

The distribution model of the data must be identified so the proper divisor to obtain one standard uncertainty, or 1σ, can be used. The distribution of the data is typically normal, also known as Gaussian, student t, or rectangular. Most calibration certificate data can be assumed to be normal and the divisor derived from the confidence level (ie. 95% = 2σ, so divisor is 2). Control chart or validation data may be normal or a student t distribution, with the former requiring 100 data points, but typically has a divisor of 1. The student t table, Figure 10.5.4, should be consulted to expand the uncertainty when the largest uncertainty contributor is of this distribution. If the distribution of the data is unknown it can be considered rectangular with a divisor of square root of three.

Since the results of forensic alcohol analysis are the mean of two results the highest contributor to the uncertainty is divided by the square root of two to determine the standard uncertainty.

The fifth step is to perform the calculation to obtain the standard uncertainties.

In the sixth step the standard uncertainties are combined. If any standard uncertainty is less than one third of the highest uncertainty then it can be removed due to it having such a small effect on the final uncertainty. The remaining standard uncertainties are reviewed to determine if any are correlated, if they are then they can be cancelled out, but typically at this point all uncertainties are uncorrelated. All uncorrelated uncertainties are combined using the root sum of squares equation.

The seventh step is to express the expanded uncertainty. Based on the confidence level of 99.8% the coverage factor should be determined using either the normal distribution value (k=3) or the student t table, Figure 10.5.4, value. The combined uncertainty is then multiplied by the coverage factor to determine the expanded uncertainty. Figure 10.5.5 shows an example of the Measurement Uncertainty Form, TF202.10.5, completed.

Once the analyst has completed the calculation the Measurement Uncertainty Form along with any attachments is reviewed by the Quality Manager for approval.

n-1	95% Confidence Level	99.8% Confidence Level	n-1	95% Confidence Level	99.8% Confidence Level
1	12.7	318.3	16	2.1	3.7
2	4.3	22.3	17	2.1	3.6
3	3.2	10.2	18	2.1	3.6
4	2.8	7.2	19	2.1	3.6
5	2.6	5.9	20	2.1	3.6
6	2.5	5.2	30	2.0	3.4
7	2.4	4.8	40	2.0	3.3
8	2.3	4.5	50	2.0	3.3
9	2.3	4.3	60	2.0	3.2
10	2.2	4.1	70	2.0	3.2
11	2.2	4.0	80	2.0	3.2
12	2.2	3.9	90	2.0	3.2
13	2.2	3.9	100	2.0	3.2
14	2.1	3.8	∞	2.0	3.1
15	2.1	3.7			

Figure 10.5.4 Student t Table

MEASUREMENT UNCERTAINTY DETERMINATION FORM

Measurand Statement (what is the quantity being measured) Quantitation of Ethanol in Whole Blood		
Traceability Statement (how it is established for the measurement) Traceability is established through external calibration of all instruments used, internal validation of calibration standards or use of certified reference standards, and monitoring quality control data using control charts.		
Technical Procedure (w/ Section, if applicable) TP101, FAA SOP	**Analyst** A. Hutson	**Date** 12/2/2013
Equipment Used for Measurement (list all equipment considered in uncertainty estimation) Hamilton Diluter, Model MC210P, S/N MD91JK5132; Thermo Fisher Trace GC, S/N XXXXXXX; External calibration standards Cerilliant (see attachment for list of Lot numbers)		

Sources of Uncertainty	Type A or Type B?	Std Dev (units)	Distribution Model	Divisor	Std Uncertainty (1σ)	Significant (Y/N)	Uncertainty Data From?
Reproducibility (C150, n=38)	A	1.50%	Student t	Sqrt 2	1.06%	Y	Control Charts
Calibration (Cerilliant, E-100)	B	0.40%	Normal	2	0.20%	N	Cerilliant certificate
Volume (sample)	B	0.18%	Normal	2	0.09%	N	Diluter calibration
Volume (calibrator)	B	0.18%	Normal	2	0.09%	N	Diluter calibration
Volume (internal std)	B	0.18%	Normal	2	0.09%	N	Diluter calibration

Equation for Combined Uncertainty ($U_{combined}$) **U combined = √{(1.06)^2} = 1.06%**			
Combined Uncertainty ($U_{combined}$)	**1.06%**		
Confidence Level	**99.8%**	**Coverage Factor (k)**	**Student t (n=30) =3.4**
Expanded Uncertainty (=$U_{combined}$ * k)	**=1.06*3.4 = 3.604 rounded to 4%**		
Date for Review 06/25/2014	**Quality Manager Approval** //signed//		

Figure 10.5.5 Example Measurement Uncertainty Form

10.5.2 Reporting Uncertainty

The last step of the simplified GUM approach is reporting uncertainty with the mean result. The uncertainty is only reported to three digits, is always rounded up during the expanded uncertainty calculation, and has the same units and precision of the mean

result. The confidence level of 99.8% is included when reporting the uncertainty with the mean result.

The uncertainty in the example above is calculated as a percentage so this is multiplied by the mean result to obtain the uncertainty associated with that specific result in the appropriate units. The mean result will be in the format x ± y at 99.8% confidence, where x is the result and y is the uncertainty. Forensic alcohol analysis final results will be reported as the truncated mean to two digits followed by the mean result out to three digits with the uncertainty and confidence level. An example of a reported result:

The final reported alcohol concentration is 0.12 gram of ethanol per 100 milliliters of whole blood.

This final result is the derived from two (or more) analyses with a mean of 0.124 ± 0.005 gram of ethanol per 100 mL of whole blood at a coverage probability of 99.8%.

Refer to Measurement Traceability and Uncertainty SOP, QP102.4.6.
Refer to Measurement Uncertainty Form, TF202.10.5.

11.0 Calculations

11.1 Identification

Ethanol and the internal standard (n-propanol) are identified based on their retention times. Retention times for ethanol and the internal standard (n-propanol) in the calibrators, controls and samples must be within ±2% of the retention time obtained from the individual volatile run and performance check standard.

11.2 Calibration Curve

The gas chromatograph is calibrated at the beginning of each run. A new calibration curve is created using standards of known value and all results are calculated automatically by the software using linear regression of the five point calibration curve based on peak area measurement.

11.2.1 Calibration

The instrument software is set to a linear regression (1st order) calibration utilizing the five calibration points and ignoring the origin (0,0). To compute the calibration curve, the software performs a regression calculation using the data points for all five calibration levels. The goal of the regression is to find the linear equation which minimizes the distance between the data points and the proposed line. Put another way, the distance from each data point to the proposed curve is called a residual, and the purpose of the regression is to minimize the set of residuals for the entire set of data. The squared residuals are summed to get a general estimate of how far away from the line the data points are, or:

$$\text{regression} = \Sigma\ (\text{residual})^2$$

In performing the regression, the data system chooses an equation which minimizes the regression value. The best least-squares approximation obtained from the regression is a polynomial of the following form:

$$y = c_0 + c_1x + c_2x^2$$

The curve coefficients, c_1 and c_2, are stored in the method. The curve coefficients define the calibration curve and constitute all the information necessary to plot the calibration curve and quantify unknown amounts of the component.
For each analytical sequence, the software generates a fit analysis, which shows a graphic representation of the calibration curve, the polynomial determined to provide the best fit to the data for the analytical sequence, and the R^2 value (showing the fit of the curve to the data). The closer the R^2 value is to the number 1 or 100% (depending on the software used), the more linear the curve.

11.2.2 Calculation

Peak area integration is performed automatically by the software. The software is programmed to calibrate with five alcohol standards. One aliquot of each standard is analyzed and set as a data point on a graph for the calibration curve. The y-axis is the "area ratio" and the x-axis is the calculated amount. The area ratio represents the area of

the ethanol peak divided by the area of the internal standard peak. The calculated amount represents percent weight per volume (% W/V) or grams of ethanol/100 mL. Since the volume and concentration of the internal standard remains constant and the dilution factor is constant, then the only variable is the amount of ethanol contained in the sample.

The following equation is utilized when manually calculating the % (W/V) of ethanol:

$$\%(W/V) = \frac{\left(\frac{U_{SamplePeakArea}}{U_{I.S.PeakArea}}\right)}{\left(\frac{K_{SamplePeakArea}}{K_{I.S.PeakArea}}\right)} xK_{EtOH\%}$$

where $U_{SamplePeakArea}$ represents the unknown sample peak area

$U_{I.S.PeakArea}$ represents the unknown internal standard peak area

$K_{SamplePeakArea}$ represents the known (e.g. calibrator information) peak area

$K_{I.S.PeakArea}$ represents the known internal standard peak area and

$K_{EtOH\%}$ represents the known % (W/V)

11.3 Requirements for the Calibration Curve

In order for the results of a run to be acceptable, the calibration curve must meet the following criteria:

1) Calculated values of calibration solutions of < 0.10% (W/V) are within ± 0.005 % (W/V) of the known values
2) Calculated values of calibration solutions of ≥ 0.10% (W/V) are within ± 5 % of the known values
3) The residual (R^2) value of the calibration curve is > 0.995 or 99.5%.

11.4 Requirements for Internal Standard Areas

11.4.1 Relative Standard Deviation

The relative standard deviation (%RSD) of the internal standard areas for a run must be < 10% for the run to be acceptable. The %RSD is obtained by dividing the standard deviation by the mean value then multiplying by 100:

$$\%RSD = \frac{S}{\bar{x}} x100$$

where S represents the standard deviation
$\bar{x}$ represents the mean of the internal standard areas

The mean ($\bar{x}$) is calculated by summing the individual results and dividing the sum by the number (n) of individual values:

$$\bar{x} = \frac{x_1 + x_2 + x_3 + x_4 \ldots}{n}$$

The standard deviation (S) is a measure of how precise the average is or how well the numbers agree with each other.

$$S = \sqrt{\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + (x_3 - \bar{x})^2 + \ldots}{n-1}}$$

11.5 Requirements for Reporting Results

11.5.1 Final Reported Result
The final reported results is the truncated mean of the individual results to the second decimal place.

$$\%BAC = \frac{x_1 + x_2 + x_3 + x_4 \ldots}{n} = \frac{0.133 + 0.136}{2} = 0.1345 = 0.13$$

For example:
The final reported result is 0.13 %

11.5.2 Reported Mean ± Uncertainty of Measurement (UOM)

The reported mean is the mean of the individual results rounded to the third decimal place. When the fourth decimal place in the mean is an even 5, round the third decimal to the nearest even number, to eliminate rounding bias. From the example above:

The reported mean is 0.134 %.

The reported UOM is a product of the reported mean multiplied by the current expanded uncertainty for the method. The UOM is reported to the same significance as the reported mean (e.g. to the third decimal) place and is always rounded up when the fourth decimal is ≥1.

For example, if the combined uncertainty ($U_{combined}$) is 4%:

UOM = 0.134 * 0.04 = 0.0053 = 0.006

The reported Mean ± UOM is 0.134 ± 0.006.

11.5.3 Determining Outliers

When a data point is suspected of being an outlier, the Dixon's Q-test is used at 98% confidence to determine whether it is to be used in the overall data set to determine the mean. This test allows us to examine if one (and only one) observation from a small set of replicate observations (typically 3 to 10) can be "legitimately" rejected or not.

The statistic experimental Q-value (Q_{exp}) is calculated. This is a ratio defined as the difference of the suspect value from its nearest one (gap) divided by the range of the values:

$$Q_{exp} = \frac{gap}{range}$$

The obtained Q_{exp} value is compared to a critical Q-value (Q_{crit}) found in tables. This critical value should correspond to the 99% confidence level.

If $Q_{exp} > Q_{crit}$, then the suspect value can be characterized as an outlier and it can be rejected, if not, the suspect value must be retained and used in all subsequent calculations.

N	Q_{crit} (CL:90%)	Q_{crit} (CL:95%)	Q_{crit} (CL:99%)
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568

Figure 11.5.1 Table of critical values of Q

11.6 Peak Resolution

11.6.1 Determining Peak Resolution

The resolution (R_s) between peaks of two (2) components may be calculated from the expression:

$$R_s = \frac{1.18\,(t_{R2} - t_{R1})}{w_{h1} + w_{h2}}$$

$$t_{R2} > t_{R1}$$

where t_{R1} and t_{R2} represent the retention times of the two adjacent peaks
w_{h1} and w_{h2} represent the peak widths at half-height.

A resolution of greater than 1.5 corresponds to baseline separation.

12.0 Reporting

12.1 Reviewing the Results

Once analysis is complete the analyst will manually transfer data from the XCaliber generated spreadsheet to the template run summary sheet. Chromeleon software automatically generates a run summary sheet. Run summary sheets generated from a manual data transfer will be randomly selected for review to check for data transfer errors at least once per quarter. Reviewer will annotate on the run summary sheet that the review has been completed and initial and date. A copy of the run summary sheet will be provided to the Quality Manager.

Prior to reporting results of an analysis, the analyst will:

1) Review the retention times for each standard, control and sample on the chromatograms to confirm retention times meet acceptance criteria of ± 2% of the known value.
2) Initial on the "Chromatograms Review by" line that this check is complete.
3) Confirm that the R^2 value is > 0.995 or 99.5% and write it on the line for R^2 on the run summary sheet.
4) Check the run summary for the following specifications:
    a) Standard values < 0.100% (W/V) are within ± 0.005% (W/V) of the known value
    b) Standard values ≥ 0.100% (W/V) are within ± 5% of the known value.
    c) The quality control standard value is within ± 0.010% (W/V) of the known value.
    d) The negative controls have a value of less than 0.005% (W/V) or "Not Found"
    e) The %RSD of the internal standard areas must be less than 10%, record it on the applicable line on the run summary sheet
5) Sign and date the "Analyst Review by" line to confirm to all requirements are met.

12.2 Technical Review of the Results

Technical review of a run is performed by someone trained and authorized to perform the review other than the analyst who diluted and analyzed the run. The technical reviewer will:

1) Review the retention times for each standard, control and sample to confirm retention times meet acceptance criteria of ±2% of the known value.
2) Initial on the "Reviewer" line to confirm that this check is complete.
3) Confirm that the R^2 value is > 0.995 or 99.5%.
4) Check the run summary for the following specifications:

a) Standard values < 0.100% (W/V) are within ± 0.005% (W/V) of the known value
b) Standard values ≥ 0.100% (W/V) are within ± 5% of the known value.
c) The quality control standard value is within ± 0.010% (W/V) of the known value.
d) The negative control has a value of less than 0.005% (W/V) or "Not Found"
e) The %RSD of the internal standard areas must be less than 10%

5) Sign and date the "Technical Review by" line to confirm to all requirements are met.

12.3 Reporting the Results

Each sample is analyzed twice on two separate runs that must meet the above criteria. The analyst will:

1) Record the results of duplicate (or more) analyses in the Sample Information Log
2) Confirm that the results:
   a) Agree within ± 5% for samples ≥ 0.100% (W/V)
   b) Agree with in ± 0.005% (W/V) for samples < 0.100% (W/V)
3) Calculate the mean to the third decimal, use rounding rules from 11.5.2.
4) Calculate the uncertainty of measurement (UOM) to the fourth decimal using the current measurement uncertainty calculations, refer to 11.5.2 for rounding rules for UOM
5) Report the mean and UOM to third decimal (same significance as the mean)
5) For the final report result, truncate the third decimal of the mean
6) Record the final result in the Sample Information Log to the second decimal.

If results do not agree within this range, additional analyses are performed. If the two results do not meet the above criteria and are between 0.006% - 0.010% (W/V) apart for results <0.100% (W/V) and are 0.01% - 0.02% (W/V) apart for samples ≥ 0.100% (W/V), then a third analysis is required. If the two results do not meet this criteria and are greater than 0.02% (W/V) apart, then a third and fourth analysis are required.

All data obtained will be included in the mean if statistical analysis shows no result is an outlier with 99% confidence. Outliers will not be included in the mean result. The analyst will document inclusion and/or exclusion of additional analyses in the Sample Information Log.

Results of less than 0.010% (W/V) or the lowest calibration standard are reported as "0.00%". The limit of quantitation for this method is the lowest calibration standard.

The reporting limit for this method is defined to be 0.010% (W/V). Results of greater than the value of the highest calibrator are reported as greater than that defined value.

The chemical analyst and technical reviewer review each result determined for all analyses to ensure that the above criteria are being met. If a discrepancy is discovered, the analyst is required to take action per Section 10.2.

12.4 Preparing the Report

A report is created using the Laboratory Report Template – Forensic Alcohol Analysis, QD005. The chemical analyst fills out all pertinent information from the Sample Information Log or confirms that it was entered correctly into the report template. Any information that is not applicable is labeled as N/A. See Reporting Results SOP, QP102.10, for specific verbiage to be used.

12.5 Administrative and Technical Review of the Report

Administrative review of a report is performed by someone trained and authorized to perform the review other than the analyst who prepared the report. The administrative reviewer will:

1) Confirm the technical review of each run is completed
2) Confirm the results from the run summary sheets match the results listed in the Sample Information Log
3) Check that the report information matches that in the Sample Information Log for each sample
4) Check the report information against the information submitted on the Request for Examination form:
   a) Confirm submitting agency name and case number
   b) Subject name
   c) Type of Offense
   d) County of Offense
5) Confirm that the two results for each sample:
   a) Agree within ± 5% for samples ≥ 0.100% (W/V)
   b) Agree with in ± 0.005% (W/V) for samples < 0.100% (W/V)
6) If additional analysis were performed, confirm outlier calculation and proper inclusion and/or exclusion of results for mean calculation.
7) Confirm the final reported result (to the second decimal) is the truncated mean
8) Confirm the un-truncated mean and measurement uncertainty, rounded up to three decimals, are reflected accurately on the report
9) Initial the "AR by" line and date below it

12.6 Finalizing the Report

Once the technical and administrative review is completed, the final report is signed by the analyst in the presence of the notary who notarizes the report.

12.7 Release and Distribution of the Report

The final report is released according to Quality System Manual, QD001, Section 2.10. The report is distributed according to the List of Recipients and Distribution Guidelines, QD010.

13.0 Maintenance

13.1 Preventive and Routine Maintenance

13.1.1 Gas Chromatograph (GC)

Routine preventive maintenance is performed on the GC according to the Maintenance Plan, TP103. An annual preventive maintenance is scheduled with a Thermo Scientific Service Engineer. All preventive and scheduled maintenance is recorded in the respective maintenance log. Logs are kept in the GC/MS Maintenance binder in the laboratory until they are archived electronically. Completed pages of the maintenance log are maintained per the Quality and Technical Records SOP, QP101.13.

13.1.1.1 Individual Volatile Retention Times and Elution Order

Analyze each of the volatiles that are found in the performance check standard individually followed by the performance check standard. Identify the retention time of each volatile from the individual runs and locate them on the chromatogram of the performance check standard to establish elution order. Record elution order and retention times on the Individual Volatile Retention Time Determination form, TF201.6. Store forms in the Alcohol Standards binder located in the laboratory until they are archived electronically. A technical review is performed for each determination. Elution order and retention times are established when new standards are prepared, the column length is altered, or operational parameters are changed.

13.1.1.2 Accuracy and Precision Run

Upon the completion of any maintenance to the GC, an accuracy and precision (A&P) run must be completed. An A&P run consists of 10 replicates of the ~0.08% linearity check standard run as if they were test samples. All results must agree within ±0.005% (W/V) of the known value in order for the A&P runs to be acceptable. The Performance Check Standard is reviewed to evaluate retention times, elution order and separation of peaks. If these criteria are met, the GC is placed back into service for forensic alcohol case samples. Annotate completion of A&P on the respective maintenance log.

13.1.2 Diluter, MicroLab 530B

At the end of each day of casework, sample a bleach solution into the dilutor tip and let stand at least 10 minutes before flushing out. Other maintenance or repairs are made as necessary. All diluter maintenance is recorded in the Dilutor Maintenance Log, TF201.3. Logs are kept in the Balance and Diluter binder in the laboratory until they are archived electronically. Completed pages of the maintenance log are maintained per the Quality and Technical Records SOP, QP101.13.

13.2 Performance Checks and Calibrations

13.2.1 Diluter, MicroLab 530B

The purpose of the performance check is to regularly check the accuracy of the diluter. The following procedure is to be performed once per month.

1) Fill a clean, dry, 1L Erlenmeyer Flask with ultra-pure water.
2) Replace the Internal Standard flask with the 1L ultra-pure water flask.
3) Set the diluter on its purge cycle, and purge 20 cycles of ultra-pure water through the lines.
4) Weigh a clean, dry flask (or beaker) and record the weight in the Diluter Calibration Log.
5) Dispense some ultra-pure water directly from the ultra-pure water dispenser into a plastic disposable cup.
6) Set up the diluter on its dispense cycle, the same setting that is used for analysis, as described in the Forensic Alcohol Section Manual, section 8.2.2.
7) Dispense 10 aliquots of the ultra-pure water from the plastic cup into the previously weighed flask (or beaker). Dispensing in a similar fashion to that done during analysis, wiping the tip before dispensing, and flushing the line into a glass beaker after each dispense.
8) Weigh the flask containing the 10 aliquots of ultra-pure water and record the weight in the Diluter Performance Check Log.
9) Subtract the original weight of the empty flask from the weight of that above, which gives you the weight of the ultra-pure water only. Record the weight in the log.
10) Determine the mean weight per aliquot and record in the Diluter Performance Check Log.

The mean weight per aliquot should be within +/-2% of the target weight of 2.00g. If the results do not fall within this range, the performance check is repeated once. If the results still do not meet the criteria, then the diluter is removed from service to be calibrated and the nonconformance is reported to the Quality Manager. The nonconformance is addressed using the Nonconforming Work SOP, QP101.9. All performance checks are recorded in the Diluter Performance Check Log, TF201.4. Logs are kept in the Balance and Diluter binder in the laboratory until they are archived electronically. Completed pages of the log are maintained per the Quality and Technical Records SOP, QP101.13.

13.2.2 Analytical Balances

13.2.2.1 Monthly Performance Check

The purpose of the performance check is to regularly check the accuracy of the analytical balance used to prepare standard solutions for forensic alcohol analysis. The following procedure is to be performed once per month.

1) Turn the balance on, make sure that it is level.
2) Zero the balance.
3) Using a NIST traceable weight set, weigh the applicable weight(s) for that balance from the set.
4) Record the measured weight in the Balance Performance Check Log, TF202.10.1.
5) Compare the measured weight to the target weight

Each weight must measure within +/- 2% of the target weight. Refer to Table 13.1 for appropriate check weights and specific acceptance criteria. If the results do not fall within this range, the analytical balance is removed from service to be calibrated and the nonconformance is reported to the Quality Manager. The nonconformance is addressed using the Nonconforming Work SOP, QP101.9. All performance checks are recorded in the Balance Performance Check Log, TF202.10.1. Logs are kept in the Diluter and Balance binder in the laboratory until they are archived electronically. Completed pages of the log are maintained per the Quality and Technical Records SOP, QP101.13.

13.2.2.2 Daily Performance Check

Each day a balance is used it is verified using one mid-range NIST traceable weight standard.

1) Follow the procedure outlined in 13.2.2.1 using only one mid-range NIST traceable weight standard. Refer to Table 13.1 for a sampling of appropriate check weights and specific acceptance criteria.
2) If a result from the performance check is outside of the acceptable range, first ensure that the balance is level and clean prior to rechecking.
3) If applicable, use the internal calibration function of the balance prior to rechecking.
4) If a result is outside of the acceptable range after performing the actions found in steps 2 and 3, the balance is immediately taken out of service until maintenance and/or calibration are performed by an approved vendor. The nonconformance is reported to the Quality Manager.

All daily performance checks are recorded in the Daily Balance Performance Check Log, TF202.10.4. Logs are kept in the Diluter and Balance binder in the laboratory until they are archived electronically. Completed pages of the log are maintained per the Quality and Technical Records SOP, QP101.13.

Table 13.1 Balances and Appropriate Check Weights

Balance Type	**Balance**	**Check Weights**
Analytical Balance	Mettler Toledo XS603S	0.100 g (± 0.002 g) 1.000 g (± 0.020 g) 10.000 g (± 0.200 g) 100.000 g (± 2.000 g) 500.000 g (± 10.000 g)
Top-loading Balance	Denver SI-4002	1.00 g (± 0.02 g) 10.00 g (± 0.20 g) 100.00 g (± 2.00 g) 500.00 g (± 10.00 g) 1000.00 g (± 20.00 g)

14.0 References

14.1 Statutes

14.1.1 North Carolina General Statutes 20-139.1
14.1.2 North Carolina General Statutes 7A-304(a)(8)

14.2 Laboratory Manuals

14.2.1 North Carolina State Bureau of Investigation, Drug Chemistry Procedure Manual, Determination of Alcohol and Volatiles in Body Fluids and Other Dilute Solutions by Headspace Gas Chromatography. (ASCLD/LAB Accredited)
14.2.2 Orange County (CA) Sheriff-Coroner Department, Forensic Science Services, Forensic Alcohol Section Manual (ASCLD/LAB Accredited)
14.2.3 Virginia Division of Forensic Science, Toxicology Technical Procedures Manual, Alcohols by Headspace GC (ASCLD/LAB Accredited)

14.3 Journal Articles

14.3.1 Chromatographic Methods for Blood Alcohol Determination, Tagliaro et.al., 580 Journal of Chromatography 161-190 (1992)
14.3.2 Determination of Ethanol in Biological Samples by Head-Space Gas Chromatography, Molina et.al., 10 Journal of Pharmaceutical & Biomedical Analysis 1069-1071 (1992)
14.3.3 Rapid Vapor Phase Method for Determining Ethanol in Blood and Urine by Gas Chromatography, Wallace et.al., 46 The American Journal of Clinical Pathology 152-154 (1966)
14.3.4 The Stability of Ethanol in Human Whole Blood Controls; An Interlaboratory Evaluation, Dubowski et.al., 21 Journal of Analytical Toxicology 486-491 (1997)

15.0 Appendices

There are no appendices for this procedure.

16.0 Revision Table

Revision #	Effective date	Revised by	Description of Revisions
Original	08/01/2009	B. Pridgen	
#1	12/01/2009	B. Pridgen	Removal of references to Laboratory Manager. Modification of text throughout.
#2	03/02/2011	B. Pridgen	Addition of Training Module
#3	11/13/2012	B. Pridgen	Reformatted entire document. Rearranged sections to comply with QSM & QP101.13. Removed repetitive text that is in QSM. Added more detail to tech/admin review. Changed verb tense throughout.
#4	03/14/2013	B. Pridgen/ A.Hutson	Addition of IS and additional analyses acceptance criteria, Section 10.4, Re-training, daily and monthly Balance checks and acceptance criteria table; Updated instrument and equipment list
#5		A. Hutson/ B. Pridgen	Addition of new instrument, change to external QC schedule, correction of typos, addition of UOM, court testimony training, secondary standard value determination criteria, chromatogram resolution criteria, training activities, %RSD for IS Areas and removal of IS Variance

Authorization

This Standard Operating Procedure, Revision Issue #5, has been approved and authorized by:

Bethany P. Pridgen, MFS Forensic Lab Manager	Date
Ralph M. Evangelous Chief of Police	Date