DOCUMENT STRUCTURE

SECTION 1: INTRODUCTION

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

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

SECTION 2: EVIDENCE HANDLING

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

Evidence is stored in a locked refrigerator at all times except during analysis. During analysis the evidence must be secured in the locked blood alcohol laboratory, if the analyst is not present.

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

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

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

Outer seals must be resealed after analysis with tamper-proof evidence tape. There should be some means of identifying when and by whom this was performed, usually by initialing and dating the evidence tape. Samples that require toxicology testing should be packaged for shipment to Washington State Patrol Toxicology Laboratory. The outer packaging and any additional tubes should be resealed and returned to the evidence section along with all samples that do not require toxicology testing.

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

SECTION 3: DILUENT, CALIBRATION STANDARDS AND CONTROLS

Internal Standard Diluent

Chemicals that meet American Chemical Society (A.C.S.) specifications will be used when available. Mix 0.2 mL of n-propanol in 2 liters of deionized water. Solution can be stored at room temperature and expires in 3 months.

A newly prepared internal standard diluent is deemed suitable for use in casework when only one integrated peak is in the negative control and the mass spectrum identifies n-propanol when compared with a reference spectrum. The internal standard diluent's preparation is documented in the blood alcohol discipline's reagent log.

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

1. The chromatogram of the negative control showing only n-propanol
2. The mass spectrum of n-propanol

Calibration Standards

NIST traceable aqueous ethanol standards at 0.020, 0.100, 0.200 and 0.500 g/100 mL are used for calibration. Calibration standards are purchased from an ISO 17025 certified supplier. A critical supply vendor form is completed for each calibration standard. The Certificates of Analysis for all calibration standards are kept in the blood alcohol laboratory.

Prior to using a new lot of calibration standard one vial from the lot should be run as a sample (in duplicate) to verify the lot falls within the manufacturer's specifications. A new shipment of the same lot does not require additional testing. The lot numbers and expiration dates should then be entered into the LIMS where they will become part of each case file as a permanent record of the standards used for each calibration.

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

1. Copy of the Sequence table for the run
2. ETOH custom Chemstation report of the controls and calibrators for the run
3. ETOH custom Chemstation report of the calibration standard duplicates being verified

Controls

1. Negative Control (Blank): Deionized Water

2. Mixed Volatiles Control: Chemicals that meet American Chemical Society (A.C.S.) specifications will be used when available. To a 500 mL volumetric flask add 500 μL of methanol, acetone, ethanol and isopropanol and 250 μL acetaldehyde. Dilute to volume with deionized water. The solution can be stored at room temperature and expires in 1 year.

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

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

   1. The chromatogram of the mixed volatile control showing baseline separation of all components
   2. The mass spectrum of each component

3. Whole Blood Ethanol Controls: Whole blood ethanol controls are purchased from a reputable supplier. A critical supply vendor form is completed for the whole blood controls. The manufacturer control sheets for all whole blood controls are kept in the blood alcohol laboratory.

   Prior to using a new lot of whole blood controls or a new shipment of a previously received lot, each vial of the received lot should be analyzed once (in duplicate) to determine the lab parameters of precision. The lab's parameters of precision are defined as +/- 0.005 or 5% whichever is greater of the mean analyzed value. A WBC worksheet template is utilized to calculate the labs parameters of precision. This worksheet will be technically reviewed by another competent analyst prior to certifying the lot. The technical review will include verifying the analytical values are correctly entered into the form and that the formulas being used by excel are pulling the correct cells for that calculation. The technical reviewer's initials and date on the lower corner of the WBC worksheet indicate that the technical review was performed. These values are then checked for agreement with the expected range by the manufacturer, if provided. The values, lot numbers, and expiration dates must be entered into the LIMS where they will become part of each case file as a permanent record of the control used for each batch analysis.

After determining a whole blood control's parameters of precision, the following documentation is included in the Whole Blood Controls binder:

1. The manufacturer control sheet
2. Whole Blood Control Spreadsheet
3. Copy of the Sequence table for the run
4. ETOH custom Chemstation Report for the controls and calibrators for the run
5. ETOH custom Chemstation Report for all the whole blood controls being verified

4. Aqueous Ethanol Controls: NIST traceable aqueous ethanol standards at 0.025 and 0.300 g/100 mL are used as low and high level ethanol controls. Aqueous ethanol controls are purchased from an ISO 17025 certified supplier. A critical supply vendor form is completed for each ethanol control. The Certificates of Analysis for all aqueous ethanol controls are kept in the blood alcohol laboratory.

   Prior to using a new lot of aqueous ethanol controls one vial from the lot should be run as a sample (in duplicate) to verify the lot falls within the manufacturer's specifications. The lot numbers and expiration dates should then be entered into the LIMS where they will become part of each case file as a permanent record of the standards used for each calibration.

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

   1. Copy of the Sequence table for the run
   2. ETOH custom Chemstation report of the controls and calibrators for the run
   3. ETOH custom Chemstation report of the aqueous ethanol standard duplicates being verified

Date Documentation of Controls and Calibration Standards

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

SECTION 4: EQUIPMENT AND INSTRUMENTATION

Diluter/Dispenser

A Hamilton Microlab diluter/dispenser is used for sample dilution. The ETHANOL dilution method on the diluter/dispenser is set for a 100 µL sample with 1000 µL of internal standard diluent along with a 1000 µL wash between samples. The complete parameters for the ethanol method can be found in the maintenance and calibration binders for each of the diluter/dispensers.

Each diluter/dispenser is sent out annually for calibration and routine maintenance. Documentation of this maintenance and calibration information is kept in binders in the blood alcohol laboratory. Other maintenance and repair is performed, as needed, according to the manufacturer's recommendation and is documented in the maintenance log.

Headspace Gas Chromatograph/Mass Spectrometer

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

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

SECTION 5: ANALYSIS PROCEDURE

Sample Selection

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

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

Blood collected into different types of tubes will be considered as separate items. The analyst should use their expertise in selecting the best tube for the analysis required and his/her report should indicate only the tube being tested. Any additional tubes in the item can be indicated in the notes field of the LIMS.

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

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

Sample Preparation

Remove calibration standards, control(s), and bloods (or beverages) to be analyzed from the refrigerator and allow to warm to room temperature prior to sampling. Prepare two vials for each sample and control that is to be analyzed. Set up one vial each for the blanks, mixed volatiles control and calibration standards. A blank is run as the first sample in each sequence followed by the calibration standards. A blank and the mixed volatiles control is run following the calibration standards. A 0.300 aqueous ethanol control, whole blood control and a 0.025

aqueous ethanol control are run in duplicate immediately prior to and following a batch of case samples. The maximum number of case samples per run is 15. A sample blood alcohol sequence can be found in the instrument binder located in the instrument room.

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

Begin sample preparation by priming the diluter/dispenser with the internal standard diluent. Ensure there is sufficient internal standard diluent in the bottle to dilute the entire run. Make sure that there are no bubbles in either syringe. Select the ETHANOL program and follow the instructions as it directs you through the setup. Ensure that each sample is mixed by inverting gently several times prior to sampling. Dispense 1000 µL of internal standard diluent, along with 100 µL of sample, calibration standard, or control into the headspace vials using the diluter/dispenser. Wipe the tip of the dispenser between each sample with a Kimwipe. Cover the vials with the caps and crimp tightly onto the vials. Continue this process for all samples in the run.

Beverage and homebrew samples that contain ethanol may need a preliminary dilution before the diluter/dispenser process. These samples may be diluted 1:100 with deionized water. The dilution factor can then be adjusted by the analyst if needed based on the test results. The resulting ethanol concentration will then need to be multiplied by the dilution used, and then converted to volume of ethanol per volume of liquid by dividing by the density of ethanol. For example:

0.050 g/100 mL (ETOH concentration) x 100 (dilution factor) ÷ 0.789 g/mL(density ETOH) = 6.3 mL/100 mL (final ETOH concentration)

In this example, the beverage would be 6.3% ethanol by volume. The “proof” would be twice that, or 12.6.

Instrument Preparation

Sequences are imported to the Chemstation software using the LIMS. Instructions on sequence importation and the method parameters are located in the binder next to the instrument. Place all vials into the auto sampler tray in the order they are listed in the sequence table.

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

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

Software Usage

Sequences are imported into Chemstation using the LIMS. Instructions on sequence importation are located in a binder next to the instrument. For additional instructions on how to use the Chemstation software refer to the manufacturer's instructions.

SECTION 6: CRIME LAB REPORTS

Upon completion of the run verify that negative control or blanks demonstrate no measurable ethanol. Ensure the mixed volatiles control has baseline separation of all components and that the mass spectrum of ethanol contains the ions 31, 45, and 46. Calibration standards should read within +/- 0.005 or 5%, whichever is greater, of their expected value. The controls should test within +/- 0.005 or 5%, whichever is greater, of the mean analyzed value determined when the lot was run initially. If a blank, mixed volatiles control, calibration standard, aqueous ethanol control or whole blood control is out of range or does not meet the above requirements the run should be rediluted and rerun.

The two analyzed values for each unknown sample must agree within +/- 0.005 or 5% whichever is greater. The reported value is the average of the two analyzed values truncated to three decimal places. For measurement of uncertainty see Appendix A.

If duplicate analyzed values do not agree within +/- 0.005 or 5% whichever is greater, then the sample will be diluted a second time and analyzed. If the second dilution's analyzed values does not fall within +/- 0.005 or 5% whichever is greater, the sample may be diluted a third time. Should the analyzed values of the third dilution not fall within +/- 0.005 or 5% whichever is greater, the sample will be reported as "Sample quality insufficient for quantitation".

None Detected will be reported for analyzed values less than or equal to 0.009 g/100mL. Linearity is established from 0.020 to 0.500 g/100mL. Alcohol values greater than or equal to 0.010 and less than 0.020 g/100mL will be reported as less than 0.020 g/100mL. Samples with alcohol values over 0.500 g/100mL will be diluted and rerun.

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

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

A LIMS system is used for compiling all case and run data into each case file and generating crime laboratory reports. Instructions for importing data are located in a binder next to the instrument. After the data is imported the analyst should review the imported data and case file. When the analyst determines the report is complete and ready for review he/she should record the date in the date of completion field of the notes function in the LIMS. The analyst should then affix their electronic signature.

After reports are signed by the analyst they are forwarded to a qualified person for technical and administrative review.

At the completion of analysis the glass headspace vials should be disposed of in the appropriate biohazard container and the evidence returned to the evidence section if no other testing is required.

SECTION 7: CASE FILES

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

- The Autotune report and Tune Evaluation
- Calibration curve
- The ETOH custom Chemstation report for each duplicate
- The ETOH custom Chemstation report for the blanks, mixed volatiles control, each calibration standard, each aqueous ethanol control and whole blood control
- A copy of the sequence table for the run
- The analyst's worksheet
- The Laboratory's Request for Laboratory Services
- Chain of Custody Documentation
- The Crime Laboratory report
- Any case related correspondence

SECTION 8: PROFICIENCY TESTING

The guideline for satisfactory completion is based on the manufacturer's expected results and the average of all respondents. This means ± 10% or ± 2 standard deviations of the average of all respondents for samples. Proficiency test results are maintained by the Quality Assurance Manager.

SECTION 9: CALCULATIONS BASED ON BLOOD ALCOHOL CONCENTRATIONS

It is common for court proceedings to inquire about calculations based on a known blood alcohol result. These include the number of drinks ingested to reach a certain blood alcohol concentration or what a person's blood alcohol level might have been at a prior time. There has been research in the field of alcohol absorption and elimination and there are a variety of methods for performing these calculations. While most methods are reliable and generate similar conclusions one method was selected by this laboratory in an attempt to standardize expert testimony.

Determination of Expected Blood Alcohol Concentration

Absorption of ethanol in the body begins immediately following ingestion. Generally, about 20% of the alcohol consumed is absorbed through the stomach while the remaining 80% is absorbed through the lining of the intestines. Alcohol absorbed through the stomach and intestines enters the blood stream and begins diffusing into the body tissue immediately. The time necessary to reach the peak blood alcohol concentration after drinking ceases varies but on average is near 45-75 minutes.

The formulas used by this lab for calculating an expected blood alcohol concentration are:

Male:
(155 lb/persons weight) * (0.020) * (# of std. drinks) = Expected BAC

Female:
(155 lb/persons weight) * (0.024) * (# of std. drinks) = Expected BAC

A standard drink is defined as ½ ounce of pure ethanol, which is equivalent to (approximately):

12 oz beer
4-6 oz table wine
1.5 oz of 80 proof distilled spirits

This formula can also be used to determine the approximate number of drinks required to reach a particular blood alcohol concentration.

Retrograde Extrapolation

It is possible for blood alcohol samples to be collected significantly later than the time of the incident; however, in criminal court proceedings the blood alcohol level at the time of incident is often the more significant question. Research in the areas of alcohol absorption and elimination has allowed for qualified personnel to extrapolate backwards from a reliable blood alcohol result

and make estimations to the blood alcohol level at an earlier time. The limitation of retrograde extrapolation is that the subject must be in the elimination phase of alcohol metabolism (no longer absorbing ethanol) during the timeframe the retrograde extrapolation is being performed.

Elimination of ethanol begins immediately upon entering the blood stream. The body metabolizes about 75-90% of ethanol by converting it to acetaldehyde, then to acetic acid, and ultimately to carbon dioxide and water. Up to 10% of alcohol can be eliminated unchanged through the breath, urine, or other body fluids. Other methods of oxidation account for the remaining elimination of alcohol.

Elimination rates vary between individuals but generally do not vary much in a given individual. The elimination rates used by this lab for retrograde extrapolation are 0.010 – 0.025 g/100mL per hour with an average of 0.017 g/100mL per hour.

Providing Opinions For Court Proceedings

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

SECTION 10: TOXICOLOGY SEND OUTS

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

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

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

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

SECTION 11: REFERENCES

1. Alan Wayne Jones, Lars Andersson. Influence of Age, Gender, and Blood-Alcohol Concentration on the Disappearance Rate of Alcohol from Blood in Drinking Drivers. Journal of Forensic Science, Vol. 41 (6): 922-926 (1996).
2. Robert B. Forney Jr. Pharmacology of Alcohol. Handout from The Robert F. Borkenstein Course on Highway Safety. May 2006.

SECTION 12: ABBREVIATIONS

AEC: Aqueous Ethanol Control

FID: Flame Ionization Detector

GC: Gas Chromatograph

HSS: Headspace sampler

PFTBA: Perfluorotributylamine

MS: Mass Spectrometer

MV: Mixed volatiles

RLS: Request for laboratory services form

RPD: Relative percent difference

WBC: Whole blood control

APPENDIX A: UNCERTAINTY OF MEASUREMENT

The forensic alcohol discipline will become compliant with the Supplemental Requirements of the American Society of Crime Laboratory Directors/Laboratory Accreditation Board with respect to measurement of uncertainty when required by the accrediting body.

APPENDIX B: REVISION HISTORY

2012 R2 Page	2012 R1 Page	Location	Revision made
all	all	all	Where applicable added mass spectrometry information
all	all	all	Where applicable removed "whole blood" when referencing control solutions.
3	3	Section 2	Removed second paragraph on evidence check out from inside laboratory.
5	5	Section 3 Internal Standard Diluent	Updated to new formula.
5	n/a	Section 3 Internal Standard Diluent Second paragraph	Added "and the mass spectrum identifies n-propanol when compared with a reference spectrum" to the first sentence.
5	5	Section 3 Calibration Standards	Updated ethanol standards level used.
5	n/a	Section 3 Calibration Standards	Added statement "A new shipment of the same lot does not require additional testing."
5	5	Section 3 Calibration Standards	Updated documentation stored in the Calibration Standards binder.
6	n/a	Section 3 Calibration Standards	Added "and the mass spectra identify each component when compared to reference spectra" to the second paragraph of #2 under Controls.
6	5	Section 3 Calibration Standards	Updated documentation requirements for mixed volatiles control
7	6	Section 3 Calibration Standards	Updated documentation stored in the Whole Blood Control binder and added section of technical review of documentation.
7	n/a	Section 3 Calibration Standards	Added #4 Aqueous Ethanol Controls.
8	7	Section 4	Removed model number of diluter/dispenser.
8	7	Section 4	Updated volumes used in dilution.
8	7	Section 4	Dilution method name changed from "NaCl" to "ETHANOL".
8	7	Section 4	Removed "The diluter/dispenser should be flushed with DI water after use."
8	7	Section 4	Removed model numbers of instrument.
9	8	Section 5 Sample Preparation	Removed second sentence "Glass headspace vials should be set up in the large test tube racks."

2012 R2 Page	2012 R1 Page	Location	Revision made
9	8	Section 5 Sample Preparation	Updated Sequence order
10	9	Section 5 Sample Preparation	Updated sample and diluent volumes used in analysis.
n/a	9	Section 5 Sample Preparation	Removed the last sentence of the first paragraph "The autosampler will accommodate 70 vials.
10	n/a	Section 5 Sample Preparation	Added the statement “The maximum number of case samples per run is 15.”
n/a	9	Section 5 Instrument Preparation	Removed first two sentences of the first paragraph "The helium for the carrier and makeup gas is supplied from tanks in the vehicle exam room. The hydrogen and air for the flame are supplied by compressed gas tanks located in the instrument room. Periodically check all tanks to ensure the pressure is not below 500 psi. "
10	9	Section 5 Instrument Preparation	Removed paragraph on vial checks.
10	n/a	Section 5 Instrument Preparation	Added paragraph on mass spectrometer tuning.
12	n/a	Section 6 Crime Lab Reports	Added statement "and that the mass spectrum of ethanol contains the ions 31, 45 and 46."
12	11	Section 6 Crime Lab Reports	Removed statement about only samples bracketed by controls must be thrown out and added failing controls require redilution and rerun.
12	n/a	Section 6 Crime Lab Reports	New paragraph describing if duplicates values to not agree added as the third paragraph.
12	11	Section 6 Crime Lab Reports	Changed linearity reporting ranges.
12	n/a	Section 6 Crime Lab Reports	Added paragraph on ethanol retention time determination.
12	n/a	Section 6 Crime Lab Reports	Added paragraph on Mass Spectrum interpretation for ethanol.
14	12	Section 7 Case Files	Added Autotune report, tune evaluation and calibration curve to case file components.
19	17	Section 11 References	Removed instrument and dilutor/dispenser manuals.

2012 R2 Page	2012 R1 Page	Location	Revision made
20	18	Section 12 Abbreviations	Added Aqueous Ethanol Control (AEC), Flame Ionization Detector (FID), Gas Chromatograph (GC), Mass Spectrometer (MS) and Perfluorotributylamine (PFTBA)
21	19	Appendix A	Changed compliance date