TEXAS DEPARTMENT OF PUBLIC SAFETY

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To:	Robert Guest	**From:**	Kenneth Evans
	Attorney at Law	**Pages:**	16 (including cover)
Fax:	866 209-9785	**Date:**	08/07/08
Phone:	469 376-1000		
Re:	Open Records Request	**CC:**	

☐ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply**

Attached is a copy of our SOP for Blood Alcohol analysis and copies of our instrument maintenance record per your request in your open records request date August 3, 2008.

STANDARD OPERATING PROCEDURES

FOR

BLOOD ALCOHOL ANALYSIS

BY

GAS CHROMATOGRAPHY (GC)

ETHANOL ANALYSIS OF BIOLOGICAL SPECIMENS BY GAS CHROMATOGRAPHY (GC)

1 Scope

The ethanol content of a biological specimen can be determined by headspace analysis of a sample using an internal standard method by gas chromatograph. Additional volatile components may be qualitatively identified.

2 Safety

Universal Blood borne Pathogen Precautions should be observed. Personal protective equipment during sample preparation should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

Inoculation for hepatitis B is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment and personal protective equipment when preparing reagents and handling volatile chemicals.

3 Related Documents

- Laboratory Submission Form
- Evidence Record Sheet
- Alcohol Analysis Worksheet (LAB-BA-01)
- Batch Record

4 Equipment, Materials, and Reagents

- Gas Chromatograph with Flame Ionization Detector, data collection and reporting system, and two different chromatographic columns suitable for alcohol analysis (e.g. Restek BAC-1 and Restek BAC-2, or comparable)
- Headspace Autosampler (optional)
- Crimper
- Vials, caps, and stoppers
- Positive displacement or air displacement micropipette and tips
- Purified water
- Ethanol standard solutions (purchased or prepared)
- 0.08 Ethanol Standard (NIST Traceable; such as Cerilliant cat # E-030)
- n-Propanol working solution
- 4.3M NaCl/n-Propanol solution
- NaCl (meets or exceeds Reagent Grade)
- Saponin/Sodium azide/Sodium fluoride solution
- Volatile mixture standard

- Ground-glass tissue grinder
- Tissue homogenizer

5 Calibration

A. At least one 0.08 g/100 mL external ethanol standard will be used as a single point calibration for the instrument.

B. Any recalibration done within the batch run must be with the same ethanol standard solution used above. This recalibration must be followed by the analysis of an 0.08 g/100 mL ethanol standard.

C. The system must be calibrated for each batch of samples examined.

6 Standards and Controls

6.1 Ethanol solutions

A. At least one 0.08 g/100 mL ethanol standard, from a source or preparation different than the 0.08 g/100 mL calibration standard previously mentioned, will be analyzed as a control standard.

B. At least one ethanol standard of 0.40 or 0.50 g/100 mL will be analyzed as an unknown to verify the linearity of the alcohol versus detector response.

C. The results of the analysis of each ethanol standard above 0.10 g/100 mL must be within 10% of its expected value. The results of the analysis of each ethanol standard below 0.10 g/100 mL must be within 0.01 g/100 mL of its expected value.

1. If the result of ethanol standards is not within allowable variance of the expected value after analysis of standards and before the analysis of any case samples, the problem will be corrected and reanalysis of standards will be performed. If results continue to fail to produce acceptable results, advise the supervisor.
2. If the result of ethanol standards is not within allowable variance of the expected value after analysis of controls and samples, correct and document the problem.
3. If a standard is not within allowable variance of the expected value during the analysis of case samples, then results from case samples bracketed by acceptable standards may be used at the discretion of the analyst.

D. If the analyst determines that the failure of the controls may be instrument related, it will be documented. Consultation with a service engineer may be needed.

6.2 Non-ethanol solutions

A. Aqueous or blood matrix method blank – At least one aqueous or blood matrix method blank, which includes all non-ethanol preparation solutions, will be analyzed. Blanks should contain no detectable volatiles other than n-Propanol. If a volatile contaminant is detected, the source of contamination will be identified and corrected. The analyst must evaluate the impact of contaminants on the validity of the analytical results. The case results may be used at the discretion of the analyst.

B. Volatile mixture standard – At least one volatile mixture standard will be analyzed with each batch analysis to demonstrate that ethanol and other

volatiles are capable of being separated and identified in a mixture. Compare the retention time and peak height to a valid quality control run.

C. n-Propanol internal standard – An aliquot of an aqueous solution of n-Propanol is added to each standard, blank, and unknown, to function as an internal standard. The n-Propanol in each injection may be compared across samples in a batch to ascertain if the process was reproducible.

7 Procedure

7.1 Evidence Examination

1. If there is a valid evidentiary breath test, no further analysis is necessary at this time.
2. Examine the evidence and document any discrepancies or irregularities.
3. Mark at least the innermost specimen container(s) with the laboratory case number and analyst's initials.
4. Record characteristics of the specimen (i.e. clotted, liquid, etc.).
5. Following sample preparation and analysis, specimen container(s) should be repackaged in the original container as soon as practical. The evidence should be re-sealed in a manner that would detect tampering.
6. The evidence should be filed in an evidence storage area until its final disposition.

7.2 Sample Preparation

1. Allow specimens, standards, and reagents to equilibrate to room temperature.
2. Label analysis vials.
3. All samples and controls must be prepared with the same reagents. Add reagents to each analysis vial to contain:
   a) *An aliquot of Saponin/Sodium azide/Sodium fluoride solution, excess amount of granular salt, and n-Propanol working solution;*
   b) *An excess amount of granular salt and n-Propanol working solution; or*
   c) *An aliquot of 4.3M NaCl/n-Propanol solution.*
4. Add an aliquot of the respective specimen, standard, or reagent.

Note: With air displacement pipettes, each sample aliquot must be drawn with a new tip. With positive displacement pipettes, the tip must be rinsed in purified water thoroughly before pipetting the next sample.

a) *If blood specimens are analyzed for alcohol where the specimen exhibits a minimal amount of clotting, invert the tube several times to facilitate mixing. The fluid in the specimen vial excluding clotted blood may be sampled.*

b) *If blood specimens are analyzed for alcohol where a majority of the sample is clotted, the entire sample must be ground to a homogeneous mixture before sampling and analyzed as whole blood.*

c) For blood tubes with the blood serum separated, the fluid in the serum excluding blood cells may be sampled.

d) If urine is analyzed, the fluid in the container may be sampled.

e) If tissues (vitreous, liver, kidney, brain) are analyzed, fluid from the container is analyzed. If no fluid is available, mix 1 mL water per gram of tissue sampled and homogenize. Analyze the resulting mixture, adjusting the results for dilution. Analysis of this sample must be accompanied by analysis of the water used to verify that no ethanol is present in the water.

f) If limited sample is available for analysis, then with the normal sampling pipette add one aliquot of sample to two or more aliquots of the same type of liquid used to prepare the calibration standard in a small vial to obtain a sufficient dilution for analysis. Mix the sample. Analyze the resulting mixture, adjusting the results for dilution. Analysis of this sample must be accompanied by analysis of the diluent to verify that no ethanol is present.

5. After the addition of the last component the cap should be secured and the vial swirled to ensure proper mixing of the contents.
6. Duplicate samples must be prepared either at this time or separately.
7. It is recommended that at least one 0.08 g/100 mL ethanol standard be pipetted prior to the preparation of all specimens and one pipetted after the preparation of the last specimen to monitor the consistency of the results.

7.3 Gas Chromatography Analysis

1. Complete the Batch Log for all specimens and standards to be analyzed.
2. Perform Gas Chromatography analysis with two different chromatographic columns.
3. Chromatograms of samples will be printed and retained in the case folder. Ethanol and n-Propanol peak identification, retention time, peak area, and quantitation results must be included with the chromatograms. Chromatograms of standards and controls will be kept in a retrievable form in the laboratory.
4. Retain the instrumental parameters in the case file and/or in a retrievable form in the laboratory.

8 Interpretation

8.1 Evaluation of Results

A. Results from analysis must be available for evaluation.

B. Specification for results:

1. For samples with all values equal to or greater than 0.10 g/100 mL, the analysis results from duplicate samples must agree within 10% of each other. This 10% variation will be calculated using the highest and lowest results, truncated to no less than three decimal places, and the following equation:

[(high value – low value) / low value] X 100

2. For samples with at least one value less than 0.10 g/100 mL, the results must agree within 0.01 g/ 100 mL of each other.

C. Conversions or Correction Factors

1. If the sample is urine, it is necessary to convert the alcohol concentration results to urine alcohol concentration units for purposes of reporting. Multiply the lowest non-truncated test result (g/100 mL) by the conversion factor (0.67).
2. If the sample is blood serum, it may be necessary to convert the alcohol concentration results to whole blood alcohol concentration units. Divide the lowest non-truncated test result (g/100 mL) by the conversion factor (1.18).
3. If a dilution is performed, the requisite dilution correction is performed after the evaluation of the raw data.

D. Conditions for reanalysis

1. If the results do not meet the above specifications, the sample preparation and analysis must be repeated. If after re-analysis the results do not meet the above specification, the case must be discussed with the quality manager.
2. If the results from the re-analysis meet the above specifications, the new set of data should be used for reporting. Both sets of chromatograms will be retained in the case folder.
3. If the results exceed the high ethanol standard, the analysis may be repeated with either a diluted sample or a higher ethanol standard. Dilute the specimen in the same manner as for limited specimen amounts and reanalyze.

8.2 Reporting ethanol concentration

A. The lowest of the results obtained will be truncated to two digits and reported.

1. For blood, the results shall be reported in grams of alcohol per 100 milliliters of blood.
2. For blood serum, results shall be reported in grams of alcohol per 100 milliliters of serum. A footnote shall be included on the report:

   "The reported value of concentration of alcohol in serum has not been converted to approximate the concentration of alcohol in whole blood."
3. For urine, the results shall be reported in grams of alcohol per 67 milliliters of urine.
4. For whole tissue, the results shall be reported in grams of alcohol per 100 grams of tissue and indicate the type of sample. A footnote shall be included on the report:

   "The concentration of alcohol in tissue or biological fluid is not equivalent to whole blood and should be interpreted with caution."
5. For vitreous fluid, liver fluid, kidney fluid, or brain fluid, the results shall be reported in grams of alcohol per 100 milliliters and indicate the type of sample. A footnote shall be included on the report:

   "The concentration of alcohol in tissue or biological fluid is not equivalent to whole blood and should be interpreted with caution."

B. If less than 0.01 g/100 mL is detected (but is above the instrument limit of detection), report "Alcohol detected (less than 0.01 grams per 100 milliliters)" or "0.00".

C. If there is insufficient sample to determine the ethanol concentration, the report should indicate "Insufficient sample for analysis" or equivalent statement.

D. If the sample is unsuitable for analysis, the report should indicate "Sample unsuitable for analysis" or equivalent statement.

E. If no ethanol is detected, the report should indicate "No Alcohol Detected".

8.3 Reporting other volatile substances

A. Volatile substances detected in a sample on both columns may be reported, if they are at a significant detectable concentration.

B. Normal endogenous levels of volatiles are listed:

Endogenous Levels of Volatiles (Baselt, 2000)	
Acetone	0.001 g/100mL
Ethanol	0.00015 g/100mL
Methanol	0.00015 g/100mL
Acetaldehyde	0.00002 g/100mL
Toluene	0
Isopropanol	0
Formaldehyde	0

C. Volatile substances detected on only one column will require confirmation by GC/MS, GC with alternate parameters (e.g. elevated oven temperature or extended runtime) for detection on both columns, or other method prior to reporting.

D. In order to report a volatile, a volatile mixture standard containing that volatile or an individual volatile standard must be tested at the time of analysis.

9 Literature and Supporting Documentation

Siek, T. J. 1972. Duplicate analysis of blood ethanol by injection onto two parallel gas chromatographic columns in rapid succession. *Journal of Forensic Sciences*, Number 2, April 1972.

Christmore, D. S., R. C. Kelly, and L. A. Doshier. 1984. Improved recovery and stability of ethanol in automated headspace analysis. *Journal of Forensic Sciences*, 29:1038-1044.

Karnitis, L., and L. J. Porter. 1971. A gas chromatographic method for ethanol determination in vapors of biological fluids. *Journal of Forensic Sciences*, 318-322.

Solon, J. J. Watkins, and L. Mikkelsen. 1971. Automated Analysis of alcohols in blood. *Journal of Forensic Sciences*, 447-452.

Dubowski, Kurt M., 1977, "*Manual for the Analysis of Ethanol in Biological Liquids*", prepared for the U.S. Department of Transportation National Highway Traffic Safety Administration.

Baselt, Randall C. 2000. *Disposition of Toxic Drugs and Chemicals in Man*, 5th edition. Chemical Toxicology Institute, Foster City, California. P. 919.

King, M. E. 1993. Ethyl Alcohol (ALC) Assays: Legal applications and other common laboratory concerns. Technical Bulletin H-44490, DuPont Company.

Preparer

Dennis Hambrick Date: 03/28/2008
Alcohol Advisory Board Chair

Concurrence

Zoe M. Smith Date: 04/02/2008
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
	09/01/2001	Original Issue
	12/07/2001	Modification Change "No Ethanol Detected" to "No Alcohol Detected"
01	12/01/2002	Minor Revision Modification Section 7.1 A #1 For samples with all values equal to or greater than 0.10, the analysis .... Modification Section 7.1 A #2 For samples with at least one value less than 0.10.... Addition Section 7.1 B #1 If the results do not meet the above specifications, the sample preparation and analysis should be repeated once. Any further attempts of reanalysis should be discussed with the quality manager. Addition Section 7.1 B #2 If a re-analysis meets the above specifications, the new set of data should be used for reporting. If after re-analysis the results do not meet the above specification, the data and condition of the evidence must be discussed with the quality manager.
02	07/01/2003	Minor Revision, numbering, rearrangement of text Addition Section 5.1 A "It is recommended that at least one 0.08 ethanol control standard be pipetted prior to all case samples and one pipetted after the last case sample. The results of the first and last control standard will be compared. If the 0.08 ethanol control standards are not within 0.01 g/100 ml of its expected value, a failure may be indicated and either a pipettor and/or analysis system check performed. " Modification Section 6.2, 2 "Duplicate samples must be prepared either at this time or separately. " Modification Section 6.2, 2 (a) "Add an excess amount of granular salt or an aliquot of 4.3M NaCl Solution (or 4.3M NaCl solution containing n-Propanol) into each sample vial. (Optional)" Modification Section 6.2, 2 (b) "Add an aliquot of the n-Propanol internal standard to each vial, if the n-Propanol was not previously

Version #	Effective Date	Brief Description of Change(s)
02	07/01/2003	added as a component of the NaCl Solution." Addition Section 6.2, 3 (b) "If limited sample is available for analysis or the concentration exceeds the high ethanol control standard, then with the normal sampling pipette add one aliquot of sample to two or more aliquots of the same type of liquid used to prepare the calibration standard working solution in a small vial to obtain a sufficient dilution for analysis. Mix the sample. The diluted sample preparation is used as above." Addition Section 7.1, A "Results from two separate aliquots must be available for evaluation." Modification Section 7.1, C 3 "If a dilution is performed, the requisite dilution correction is performed after the evaluation of the raw data." Modification Section 7.2, B "If less than 0.01 g/100 ml is detected (but is above the detection limit for your instrument), report "less than 0.01" or "0.00". " Modification Section 7.3, A "Volatile substances detected in a sample on both columns should be reported, if they are at a significant concentration above normal endogenous levels." Addition Section 7.3 C "In order to report a volatile, a volatile mixture standard containing that volatile or an individual volatile standard must be tested at the time of case sample analysis for verification of the retention time." Addition to Section 8 citation for "Disposition of Toxic Drugs and Chemicals in Man".
03	12/01/2003	Major Revision Section 5 (as recommended by the advisory board) Major Revision Section 6 (as recommended by the advisory board) Modification to Section 7.1 B #2 "...the results must agree within 0.01 g/ 100 mL of each other." Addition Section 7.1 B #3 dilution correction after raw data evaluation. Modification to section 7.1 C #2 "If the results from the re-analysis meet the above specifications, the new set of data should be used for reporting. Both sets of chromatograms will be retained in the case folder." Modification to Section 7.1 C #3 "...a higher ethanol standard. Dilute the specimen in the same manner as for limited specimen amounts and reanalyze." Modification to Section 7.3 clarification that the retention time to report other volatiles must be conducted at time of analysis.

Version #	Effective Date	Brief Description of Change(s)
04	11/01/2004	Modification of Section 3 "Batch ~~Log~~ Record" Addition to Section 4 "0.08 Ethanol Standard (NIST Traceable; Such As Cerilliant Cat # E-030)" Modification to 5.1 A "At least one 0.08 g/100 mL external ethanol standard will be used as a single point calibration for the instrument. ~~All calibrations must use the current legal limit for intoxication."~~ Deletion Section 5.2 C and D Addition to Section 7.1 B 1 "...0.10 g/100 ml..." Addition to Section 7.1 B 2 "...0.10 g/100 ml..." Modification to Section 7.2 B "If less than 0.01 g/100 ml is detected (but is above the instrument limit of detection ~~limit for your instrument~~), report "alcohol detected (less than 0.01 grams per 100 milliliters)" or "0.00"." Addition to Section 7.3 A table of endogenous levels of volatiles
05	08/01/2005	Modification to Section 4, "n-Propanol ~~internal standard~~ working solution; 4.3M NaCl/n-Propanol solution; Saponin/Sodium azide/Sodium fluoride solution ~~(optional)~~" Deletion Section 6.2 #5 Modification to Section 6.2 #3, "All samples and controls must be prepared with the same reagents. Add reagents to each analysis vial to contain either (a or b): a) An aliquot of ~~an aqueous~~Saponin/Sodium azide/Sodium fluoride solution, excess amount of granular salt, and n-Propanol working solution; or~~containing saponin, sodium azide, and sodium fluoride may be added. (Optional)~~ b) ~~Add an excess amount of granular salt or~~ An aliquot of 4.3M NaCl/n-Propanol solution ~~(or 4.3M NaCl solution containing n-Propanol) into each sample vial. (Optional).~~" Addition to Section 7.3 B, "Volatile substances detected on only one column will require confirmation by GC/MS, GC with alternate parameters (e.g. elevated oven temperature or extended runtime) for detection on both columns, or other method prior to reporting."

Version #	Effective Date	Brief Description of Change(s)
06	09/15/2006	Modification to Section 4 "~~Granular~~ NaCl (meets or exceeds Reagent Grade)" Modification to Section 5 heading and separating calibration from standards and controls Modification to Section 7.2 #3 "Add reagents to each analysis vial to contain ~~either (a or b)~~:...(b) An excess amount of granular salt and n-Propanol working solution; or...." Modification to Section 8.3 A-B "Volatile substances detected in a sample on both columns ~~should~~ may be reported, if they are at a significant detectable concentration ~~above normal endogenous levels~~. Normal endogenous levels of volatiles are listed;"
07	07/20/2007	Modification Section 7.2: sample preparation with regard to the condition of submitted sample Addition to Section 8.1: C. Conversions or Correction Factors.1 If the sample is urine, it is necessary to convert the alcohol concentration results to urine alcohol concentration units for purposes of reporting. Multiply the lowest non-truncated test result (g/100 mL) by the conversion factor (0.67). 2. If the sample is blood serum, it is necessary to convert the alcohol concentration results to whole blood alcohol concentration units for purposes of reporting. Divide the lowest non-truncated test result (g/100 mL) by the conversion factor (1.18). Modification 8.2 regarding the reporting of values in different biological samples. Addition to Section 9: King, M. E. 1993. Ethyl Alcohol (ALC) Assays: Legal applications and other common laboratory concerns. Technical Bulletin H-44490, DuPont Company.
08	04/15/2008	Major revision - Sections 7.2, 8.1, and 8.2 Advisory Board 03/11/2008

INSTRUMENT

MAINTENANCE RECORD

FOR

GAS CHROMATOGRAPH (GC)

INSTRUMENT MAINTENANCE RECORD

GARLAND LABORATORY

Manufacturer:Shimadzu **Model: GC-17A** **Serial Number: C11123781698**
DPS Inventory: 387378 **Instrument #: 19**
Installation Date: 5/3/00 **Approval Date: 6/19/00**

Date	Type of Service Performed	Serviced By
~~7-23-07~~ 7-23-07	~~Changed inlet liner and o-ring~~ 7-23-07 should have been inst. 2087	7-23-07
8.10.07	Replaced columns and inlet ferrule AND detector ferules	

INSTRUMENT MAINTENANCE RECORD

GARLAND

Manufacturer: *Shimadzu* **Model:** *GC-17A* **Serial Number:** *C11123781698*

Inventory *387378* #19

Date	Type of service performed	Serviced By:
11/17/00	Replaced Detector #1. Because it was dirty Checked operation OK.	Shimadzu.

maintain