State of North Dakota)
)ss
County of Burleigh)

I, Charles E. Eder, do hereby certify that I am a duly-appointed State Toxicologist for the State of North Dakota and an official custodian of the records and files of the office thereof, that I have carefully compared the

APPROVED METHOD TO CONDUCT BLOOD ALCOHOL ANALYSIS (TxS-020) REVISION NUMBER 0.4

hereto attached with the respective original as the same appears of record on file in the Office of Attorney General, Crime Laboratory Division, in the County of Burleigh, North Dakota, and find the same to be a true and correct copy thereof and of the whole thereof. In witness whereof I have set my hand at the city of Bismarck, in said county this:

Handay of TANUARY, 2016 Charles E. Eder, State Toxicologist
State of North Dakota))ss County of Burleigh)
On this day of <u>Sanuary</u> ,, <u>Dolle</u> , before me personally appeared Charles E. Eder, known to me to be the State Toxicologist for the State of North Dakota, and acknowledged to me that he has executed the same.

Subscribed to and sworn before me on this:

04 day of January, 2016

Stacie L. Fleck-Merkel

Notary Public, State of North Dakota My Commission Expires August 16, 2017 STACIE L. FLECK-MERKEL
Notary Public
State of North Dakota
My Commission Expires Aug. 16, 2017

Notary seal/stamp

Number: TxS-020

Title: APPROVED METHOD TO CONDUCT BLOOD

ALCOHOL ANALYSIS

Distribution List: Revision Number: 0.4 ☐ Master Manual Revision of SOP replaced by this new SOP: 0.3 ☐ Toxicology Section **Effective Date:** ☐ Alcohol and Volatiles Unit Scope: This procedure is used to determine ethanol concentrations by gas chromatography with headspace sampling. Other volatile concentrations may be determined by this procedure. This procedure may be used with matrices such as blood, urine, vitreous, tissues, biological fluids and liquids. Approved By: Chala & So. Date: 04 Tun 16 Date: 4 Jan 2016

Date: 04 Jan 2016 Quality Manager: Chi Forlie

Authorized By: ___

Toxicology Section/Alcohol and Volatiles Unit Approved Method to Conduct Blood Alcohol Analysis

Version 0.0

REVISION HISTORY LOG APPROVED METHOD TO CONDUCT BLOOD ALCOHOL ANALYSIS

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Approved Method to Conduct Blood Alcohol Analysis
New Ethanol Commercial Standards Worksheet <u>TxW-042</u>
Combi-Pal Alcohol Analysis Worksheet <u>TxW-020</u>
Alcohol Analysis Instrument Logbook <u>TxW-036</u>

APPROVED METHOD TO CONDUCT BLOOD ALCOHOL ANALYSIS

SCOPE:

This procedure is used to determine ethanol concentrations by gas chromatography with headspace sampling. Other volatile concentrations may be determined by this procedure. This procedure may be used with matrices such as blood, urine, vitreous, tissues, biological fluids and liquids.

BACKGROUND INFORMATION:

Approved Method to Conduct Blood Alcohol Analysis 2 (Revised 11/03/08)

Standard Operating Procedure Blood Alcohol Analysis (Method 2) (Revised 11/03/08)

Improved Recovery and Stability of Ethanol in Automated Headspace Analysis, J. Forensic Sci. 1984 Oct: 29(4):1038-44; Christmore, DS; Kelly, RC; Doshier, LA.

PRINCIPLE:

- A. Headspace chromatography is based on Henry's Law. Henry's Law states that for a dilute solution, the solubility of a gas in a liquid expressed as a mole fraction depends upon the pressure of the gas. There is a fixed ratio between the mole fraction of the gas and the mole fraction in the liquid. This ratio remains constant for a given temperature.
- B. The diluent solution containing an internal standard is added to a blood sample, or other suitable matrix, and sealed in a headspace vial. The headspace vial is incubated at a constant temperature for a specified time. The headspace vapor above the liquid is analyzed by gas chromatography with a flame ionization detector. Ethanol and other volatiles are identified by retention time. This procedure is suitable for quantitative analysis of ethanol and other volatiles.

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SPECIMENS:

- A. Optimum sample volume is 3 mL or greater. Samples which contain less than 3 mL may be analyzed. Determination will be made by the analyst.
- B. Acceptable specimens include blood, urine, vitreous, tissues, biological fluids, or other liquids. Other specimens may be analyzed.
 - 1. Blood: The preferred sample is blood that is submitted in the ND Crime Laboratory's Blood Collection Kit. The kits have sterile tubes with sodium fluoride and potassium oxalate.
 - 2. Urine: The preferred sample is urine that is submitted in the ND Crime Laboratory's Urine Specimen Collection Kit. The kits have containers with sodium fluoride.
 - 3. Other: County Coroners are encouraged to use the ND Crime Laboratory's Post Mortem Analysis Kit. Samples submitted in suitable collection tubes and containers will be analyzed.
- C. Samples can be stored in a refrigerator or freezer.

INSTRUMENTS, EQUIPMENT, APPARATUS AND CONSUMABLES:

- A. As determined by analysts, appropriate apparatus, lab supplies, equipment, glassware, control matrices or consumables may be substituted for analytical procedures.
- B. As determined by analysts, instrument conditions such as temperature, flows, pressures and other parameters for gas chromatographs, headspace autosamplers and chromatography software may be updated to obtain optimum chromatographic analytical results. The parameters listed for any instrument are suggested initial operating conditions.
- C. Not all settings will be listed for the instruments, equipment or chromatography systems.
- D. Gas Chromatograph:
 - 1. Columns
 - 1. Restek: Rtx[®]-BAC1, Rtx[®]-BAC2 or equivalent
 - 2. PerkinElmer®: Elite-BAC1, Elite-BAC2 or equivalent
 - 3. Other vendor's columns are acceptable and may used
 - 4. Pre-columns, Y-splitters and two hole ferrules may be used
 - 2. Gas Chromatographs
 - a. FID detector or equivalent for volatiles
 - b. Capillary, Split/Splitless Injector, or equivalent
 - c. Column temperature: 30 75 °C

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- d. Gas flows:
 - 1. Hydrogen Carrier: 5 30 psi or 4 30 mL/min
 - 2. Hydrogen FID: 15 45 mL/min
 - 3. Air FID: 300 450 mL/min
 - 4. Nitrogen FID: 30 mL/min (if make-up gas is needed)
 - 5. Split: 1:1 ratio to 1:25 ratio (autosampler and GC dependent)
 - 6. Septum Purge: ~ 5 mL/min
- e. Injector temperature: 150 300 °C
- f. Detector temperature: 150 300 °C
- g. GC run time: 2 6 min
- E. Headspace Autosamplers: CombiPAL (CTC) Air-Tight Syringe System:
 - 1. Type: HS-INJ
 - 2. Syringe: 1.0 or 2.5 mL-HS
 - 3. Sample Volume: 250 1000 μL
 - 4. Incubation Temp: 60.0 75.0 °C
 - 5. Incubation Mode: Constant
 - 6. Incubation Time: 8 12 min
 - 7. Agitation: 5 10 s On, 50 55 s Off
 - 8. Syringe Temp: 70 85 °C
 - 9. Inject To: GC Inj1 or GC Inj2
 - 10. Syringe Flushing: 1 3 min (air or nitrogen gas)
 - 11. GC run time: Determined by instrument sequencing
- F. Atlas™ Chromatography Data System (CDS)
 - 1. Chromatography software settings are dependent on type of instrument used, i.e. gas chromatograph and headspace autosampler.
 - 2. Settings will be optimized and updated as needed.
- G. Headspace vials, septa and caps
- H. Vial crimper
- I. Automated Pipettor Diluter (i.e. Hamilton 500 Series)
- J. Additional laboratory equipment or supplies may be used:
 - 1. Pipettes (micropipette, e.g. SMI® or Eppendorf®)
 - 2. Repipet® or equivalent
 - 3. Weighing bottles and lids
 - 4. Analytical balances
 - 5. Volumetric flasks and stoppers (various sizes)
 - 6. Polyethylene bottles (500 mL)
 - 7. Storage vials and sealing caps (various sizes)

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- 8. Beakers (various sizes)
- 9. Transfer pipettes
- 10. Test tubes

SAFETY PRECAUTIONS:

- A. Use universal precautions according to Blood Borne Pathogen's Exposure Plan.
- B. Use appropriate safety precautions while handling chemicals and reagents. Refer to current Safety Manual.

REAGENTS, CHEMICALS, CONTROLS AND STANDARDS:

- A. Standards, internal standard solution, diluent solution, known matrices and controls prepared by any individual certified to perform blood alcohol analysis may be used by other analysts.
- B. Chemicals:
 - 1. Ethanol (ethyl alcohol), CH₃CH₂OH, 200 proof, USP grade.
 - 2. n-Propanol (1-propanol), CH₃CH₂CH₂OH, analytical grade or better.
 - 3. Acetone, analytical grade or better.
 - 4. Isopropanol (2-propanol), analytical grade or better.
 - 5. Methanol (methyl alcohol), analytical grade or better.
 - 6. Other volatiles, analytical grade or better.
 - 7. Sodium fluoride, NaF, analytical grade or better.
 - 8. Sodium hydrosulfite, Na₂S₂O₄, analytical grade.
 - 9. Ammonium sulfate, (NH₄)₂SO₄, analytical grade or better
 - 10. Water, filtered (e.g. Millipore® or equivalent)
- C. Commercial Ethanol Standards:
 - Commercial ethanol standards may be purchased in a concentration range of 0.010 g/100mL to 0.500 g/100mL. Expiration date is determined by manufacturer. Follow manufacturer's storage requirements. If not stated, store at either room temperature or refrigerate until opened. Once opened, store in refrigerator.
 - 2. New commercial ethanol standards analysis:
 - a. Standards will need to be checked against previous standards as new lot numbers of ethanol standards are acquired.
 - b. Analyze 2 sets of duplicates of each ethanol standard by gas chromatography with headspace analysis.

c. Standards < 0.100 g/100mL must be within \pm 0.005 g/100mL of the stated value, while standards \geq 0.100 g/100mL must be within \pm 5% of the stated value.

D. Volatiles Solution:

- Lab prepared volatiles solution.
 - a. The volatiles solution is a dilution of 50 μL each of methanol, acetone, ethanol and isopropanol pipetted into a 100 mL volumetric flask.
 - b. The flask is approximately half filled with filtered water before the addition of the various volatiles and then filled to the mark with filtered water.
 - c. Invert several times to mix.
 - d. Transfer to labeled laboratory vials and store in the refrigerator.
 - e. This solution is for qualitative use only.
 - f. Expiration date is 6 months from the date of preparation.
- 2. Commerical volatiles solution may be used. Expiration date is determined by the manufacturer. Follow the manufacturers storage requirements. If not stated, store either at room temperature or refrigerate until opened. Once opened, store in the refrigerator.
- E. Diluent Solution: Made with ammonium sulfate, sodium hydrosulfite, and filtered water. Store at room temperature. No expiration date.
 - 1. The diluent solution is prepared by dissolving ammonium sulfate (132 g) and sodium hydrosulfite (17.4 g) per liter of filtered water.
- F. Internal Standard Solution: Made with n-propanol and diluent solution. Store at room temperature. Expiration date is 6 months from date of preparation.
 - 1. The internal standard solution is prepared by diluting a weighed or aliquoted amount (0.2 g/L) of n-propanol with diluent solution to obtain a concentration within the range of 0.018 g/100mL to 0.022 g/100mL.
 - The actual concentration of n-propanol is not critical as long as it remains constant during a batch of samples being analyzed. Verify that an adequate amount of internal standard is available before analysis begins.
 - 3. Other volatiles may be used as an internal standard as the need arises. The chosen internal standard cannot interfere with the retention time and resolution of the ethanol peak.
- G. Commercial Controls: Concentration ranges of controls must be within the range of standards used. Expiration date is determined by manufacturer. Follow manufacturer's storage requirements. If not stated, store at either room temperature or refrigerate until opened. Once opened, store in refrigerator.

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H. Blank Blood: Prepared with whole blood (human or animal) or packed Red Blood Cells (RBCs) and adding analytical grade sodium fluoride. Prepare at a concentration of 10 mg/mL. Expiration date is 4 months from date of preparation. Store in refrigerator.

SAMPLE PREPARATION:

See SOP TxS-021 Preparation, Sampling and Disposition of Samples in Toxicology.

PROCEDURE:

- A. Preparation of Standards, Controls, Samples, Blank, and Zero (See Table I):
 - 1. Each ethanol standard is prepared in singlet. Blank, zero, and volatiles are also prepared in singlet.
 - 2. Commercial controls are prepared as needed and may be analyzed more than once. This is equivalent to one control before and one control after each set of duplicate samples.
 - 3. Case samples are prepared in duplicate. Samples may be analyzed more than once.
 - 4. Once all components are placed in a labeled vial, it is capped and crimped.

TABLE I Preparation for Analysis					
	Volume Used	Amount of Blood Added	Amount of Filtered Water Added	Amount of Diluent Added	Amount of IS Solution Added
Standards	100 μL	100 μL		The state that	2 mL
Commercial Controls	100 µL	100 μL			2 mL
Blank		100 µL	100 µL	2 mL	
Zero		100 µL	100 μL		2 mL
Volatiles	100 µL	100 µL			2 mL
Sample – Blood	100 μL		100 µL	Elle des Siles	2 mL
Sample – Urine or other	100 µL	-	100 µL		2 mL

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CLEANUP:

- A. Dispose of the bench covers and other disposable materials using current Blood Borne Pathogen exposure procedures.
- A. Clean and disinfect any used equipment and glassware that is not disposable.

ANALYSIS:

- A. Through Forensic Advantage® (FA) LIMS Batching Process, a batch worksheet is prepared indicating the position of each vial in the autosampler. If FA LIMS Batching Process is not used, a worksheet indicating the vial positions may be prepared by hand.
- B. The sequence for alcohol analysis will be to run the 5 ethanol standards, blank, zero, and volatile solutions; followed thereafter by a constant pattern of a control, sample (in duplicate), and ending with a control.
- C. The standard curve should be analyzed with a quadratic calibration model. The correlation coefficient (R) will be calculated and if the correlation coefficient is not greater than or equal to 0.9995, then the standard curve should be prepared again.
- D. Upon completion of the analysis, the position and identity of the vials should be compared to the batch worksheet to verify the injection sequence prior to the removal of the vials from the autosampler.

CALCULATIONS:

- A. Peak areas will be used for determining ethanol concentrations.
- B. The Atlas™ CDS system will be used for the following: integration, identification of the peak, calibration, quantitation, and results generation. Initial results will be displayed to four digits.

C. Ethanol concentration can be calculated with the following formula:

$$Cs = \frac{-b + \sqrt{b^2 - 4a(c - R)}}{2a} \times K$$

$$Where:$$

$$R = aCs^2 + bCs + c$$

$$R = \frac{And}{Peak Area of Ethanol}$$

$$Peak Area of Internal Standard$$

Cs = Ethanol concentration, grams per 100 mL of blood or other fluids, or grams per 67 mL of urine

K = conversion factor (1.0 for blood, 0.67 for urine, 0.85 for serum)

QUALITY ASSURANCE:

- A. The correlation coefficient (R), as determined via quadratic calibration model, of the five standards must be ≥ 0.9995.
- B. The reported concentration of all controls ≥ 0.100 g/100mL must be within ± 5% of the expected value. Controls < 0.100 g/100mL must be within ± 0.005 g/100mL of the expected value. If any control falls out of acceptable range, the case sample prior to and immediately following that control must be reanalyzed.</p>
- C. For case sample duplicates ≥ 0.100 g/100mL, the percent relative difference between the two duplicate values shall be less than or equal to 3%. If the percent relative difference is greater than 3%, then the case sample must be reanalyzed. Duplicate sample concentrations < 0.100 g/100mL need to be within ± 0.005 g/100mL of each other or the sample duplicates will need to be reanalyzed.</p>

REPORTING:

A. The ethanol concentration of each duplicate will be calculated to four digits. The lowest calculated ethanol concentration will be truncated to three digits and the three digit result will be reported (example 0.123 g/100mL) on the Toxicology Alcohol/Volatiles Analytical Report in the results section of the summary of analysis table.

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- B. Measurement Uncertainty
 - See the current version of ADM-025 Measurement Uncertainty for documentation requirements for the measurement uncertainty estimation.
 - 2. The coverage probability (level of confidence) of the estimated expanded uncertainty for ethanol concentrations will be reported at 99.73%.
 - 3. The average ethanol concentration will be calculated from duplicate 4 digit results. The average concentration will be truncated to 3 decimal places.
 - 4. The rounded expanded measurement uncertainty will be multiplied by the truncated average ethanol concentration to obtain the uncertainty. The uncertainty obtained will be rounded to 3 decimal places.
 - 5. The measurement uncertainty will be reported as the average result plus or minus the uncertainty (Example, 0.123 ± 0.010 g/100 mL) on the Toxicology Alcohol/Volatiles Analytical Report Addendum.
- C. Results below the lowest standard will be reported out as 0.000 g/100mL. No measurement uncertainty will be calculated or reported for these results.
- D. No measurement uncertainty will be reported for liquid (e.g. beverage) samples.
- E. Urine samples will be reported out after multiplying the results by 0.67. Example, 0.123 g/100mL result will be reported as 0.082 g/67mL.
- F. Serum samples will be reported out after multiplying the results by 0.85. Example, 0.123 g/100mL result will be reported as 0.104 g/85mL.
- G. If the concentration of a sample is greater than the highest standard concentration, a portion of the sample will be diluted with filtered water and then reanalyzed. The concentration of ethanol obtained by using the above procedure will be multiplied by the corresponding dilution factor to calculate the concentration of ethanol in the specimen.
- H. If the volume of the sample submitted is less than what is necessary to perform the analysis, the Toxicology Alcohol/Volatiles Analytical Report will state the quantity of specimen was not sufficient for analysis.
- I. If the sample submitted is not suitable for analysis due to sample condition, the Toxicology Alcohol/Volatiles Analytical Report will state no result obtained due to sample quality.

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- J. Atlas™ results will be imported to FA LIMS worksheets by the FA Batching Process. Atlas™ results may also be manually entered into the FA LIMS worksheets.
- K. Atlas™ reports consisting of the calibration curve, control summary, sample summary, Form 101, and chromatograms will be attached electronically in a PDF format to the FA LIMS case record.
- L. The Atlas™ result will be compared to the FA LIMS worksheet and checked for accuracy.
- M. The Toxicology Alcohol/Volatiles Analytical Report will be generated by FA LIMS.
- N. The Toxicology Alcohol/Volatiles Analytical Report Addendum for ethanol concentration measurement uncertainty will be generated by Excel spreadsheet or FA LIMS.
- A peer review of the case record will be performed before the reporting of results.
- P. As needed, a certified copy of the Submission Form (Form 104 or 104-U) and Toxicology Alcohol/Volatiles Analytical Report (including the Toxicology Alcohol/Volatiles Analytical Report Addendum for ethanol concentration measurement uncertainty if applicable) will be prepared and sent (mailed or electronic) to the submitting agency or officer.

SAMPLE DISPOSTION:

See SOP TxS-021 Preparation, Sampling and Disposition of Samples in Toxicology.

COMPETENCY TEST AND AUTHORIZATION:

- A. A competency test is required before an analyst can perform analysis on case samples. The competency test will require the following:
 - Preparing a standard curve, blank, zero, volatile, and known controls.
 - 2. Running the samples on a chromatography system.
 - 3. Demonstrate that the acceptance criteria has been met.
 - 4. Results have been reviewed by the State Toxicologist or Technical Lead Analyst.

- 5. Questions about the procedure have been asked and answered correctly.
- B. Authorization for the procedure must be documented before analysis of case samples commences.
- C. Minor changes or updates to this procedure will not require the retaking of a competency test or reauthorization to perform analysis.

FURTHER INFORMATION:

- A. All data, methods, sequences, and reports will be kept electronically on either the computer attached to the instrument, enterprise software package or the Crime Laboratory servers.
- B. If possible, reported data and appropriate parameters should be moved to a Crime Laboratory server for protection.
- C. If the instrument software package allows, paths to the file locations will appear on the printouts or reports. Note: Once data is moved to a Crime Laboratory server, the data paths may not update properly according to the original report or printout.
- D. The operating conditions for each acquisition method can be obtained from a logbook or printed from each individual instrument, enterprise software package or Crime Laboratory server.
- E. The data pertaining to a case file both electronic and hardcopy will be destroyed at the appropriate time interval.
- F. Manuals supplied electronically will be available on the instrument's attached computer and/or on a server location available to those who need the manual. Hardcopy manuals will be located near the instrument or equipment. Considerations will be taken for the manual to not become compromised from routine use and analysis.
- G. Any reference for background information made in a procedure will be available for review. The information will be attached to the SOP or will be kept in a centralized location for that discipline.

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COMMENTS:

The procedure outlined above is an approved gas chromatographic method used by the Office of Attorney General, Crime Laboratory Division, for the determination of ethanol. When the need arises, other approved methods may be used.

REVISION HISTORY READING LOG APPROVED METHOD TO CONDUCT BLOOD ALCOHOL ANALYSIS

REVISION NUMBER	DATE READ	ANALYST (Print Name)	SIGNATURE
0.0	17June11	Janelle Portscheller	Jandle Portot
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0.0	17 June Zoll	Chris Focke	Cho toche
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REVISION HISTORY READING LOG APPROVED METHOD TO CONDUCT BLOOD ALCOHOL ANALYSIS

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