AUSTIN POLICE DEPARTMENT
FORENSIC CHEMISTRY SECTION
BLOOD ALCOHOL TECHINCAL MANUAL

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1 SCOPE AND APPLICATION

The following methods are to be used to identify and quantitate ethyl alcohol (ethanol) in blood.

2 SUMMARY

Headspace Gas Chromatography is a validated and scientifically approved method for the analysis of volatiles in biological samples. A volatile substance is converted to a gas when heated (e.g. ethanol). An aliquot of the blood specimen is transferred to a vial containing an internal standard. The vial is sealed and heated, resulting in the volatile components moving from the liquid into the gas phase or headspace. At constant temperature, the volatile components will partition into the headspace above the sample based upon the partition coefficient of each substance. Under constant conditions, equilibrium will be reached between the sample phase and gas phase. At this point the concentration of substance in the headspace or gas phase is proportional to the concentration of substance in the sample, and the amount of substance in the sample may be calculated. A portion of the headspace is then introduced into the gas chromatograph. The gaseous portion is divided between two different, specially coated narrow tubes (columns). As it flows through these columns, the different components of the gaseous mixture will interact with the column coatings to varying degrees based on their different chemical and physical properties and separate as they progress through the column. A detector monitors the time it takes for a component to pass through the column (retention time). The retention time is compared to known standards on the two columns for identification. Ethanol quantitation is achieved by running ethanol standards of known concentration and creating a standard line. Comparison of the response of the blood specimen with the known standards produces an accurate determination of the alcohol concentration.

3 PRECAUTIONS

Thorough mixing to ensure that the blood specimen is homogenous and accurate pipetting are of particular importance to accurately reflect the blood alcohol levels. Failure to do so can result in widely differing results between duplicate samples.
Maintenance or service to an instrument is followed by verification by analyzing positive, negative and resolution controls.
Annual preventive maintenance is performed by the service company under contract. All other maintenance is performed on an "as needed" basis.

4 SAFETY

- The most common type of chemical or biological exposure in this type of laboratory is a splash to the skin or eye. Skin, mucous membranes, or eyes which have been splashed with commonly used chemicals or biological should be thoroughly flushed for at least 15 minutes with cool tap water or eye wash station. Report the incident immediately to a supervisor and seek medical attention as necessary. Refer to the Department Safety Manual and the appropriate MSDS for general safety and hazard information regarding biohazard materials and disposal.
- Always use universal precautions (Gloves, face protection, and lab coat).
- At completion of the sample preparation, wipe the working surfaces, pipetters, and any other equipment or surface which may have become contaminated with a biological material with a 10% solution of bleach or other appropriate disinfectant.
- The tissue grinder must be used with particular caution to insure that no blood is spilled in the work area. It must also be carefully cleaned and sterilized with a 10% bleach solution after use.

5 EQUIPMENT

- A positive displacement micro-pipetter with disposable tips and plungers capable of accurately delivering a 200 microliter sample.
- A dispenser capable of accurately delivering a 2 milliliter sample.
- Disposable 20 or 22 ml glass headspace vials, aluminum crimp tops with appropriate septa.
- A 1/8th teaspoon measuring spoon.
- A ground glass tissue grinder.

6 REAGENTS AND PREPARATIONS

- Ethanol - (not less than Reagent Grade) or commercially prepared standards with known ethanol concentrations.
- n-Propanol - (not less than Reagent Grade).
- Sodium Chloride - (not less than Reagent Grade).
- Volatile mixture solution – use a commercially prepared standard or prepare a solution as follows:
  Add the following to a 100 milliliter volumetric flask:
  - 50 milliliters deionized water
  - About 100 milligrams of acetaldehyde
  - About 100 milligrams of acetone
  - About 200 milligrams of ethanol
  - About 150 milligrams of Isopropanol (2-propanol)

- About 300 milligrams of methanol
- Bring to volume with deionized water.

This has an expiration life of one year.

Stock Solutions:

- All reagents and prepared solutions will be labeled with the date of preparation and the initials of the preparer and included in an appropriate logbook. (ASCLD 5.1.3)
- All reagents and working solutions, whether purchased or prepared, will be verified before use in casework. (ASCLD 5.1.3)
- 10% Stock Ethanol Solution: (Skip if using commercially prepared standards) Accurately weigh 10.0 grams of absolute reagent grade ethanol into a 100 milliliter volumetric flask and fill to volume with deionized water. This solution has an expiration life of one year.
- 1% Stock n-Propanol Solution:
  - Pipette between 3.1 & 3.2 milliliters of the n-Propanol into a 250 ml volumetric flask and fill to volume with deionized water.

  OR
  - Weigh approximately 2.5 grams of the n-Propanol into a 250 ml volumetric flask and fill to volume with deionized water.

    This solution has an expiration life of one year.
- Standard Working Ethanol Solutions: (Skip if using commercially prepared standards and controls)
  - Pipette the following volumes (milliliters) of the 10% Stock Ethanol Solution into separate 100 ml volumetric flasks to make the given % W/V working solutions. (These are examples, other volumes may be used and not all volumes must be used).

Volume (mL of stock) to give	% W/V (grams/100 ml)
0.10	0.01
0.20	0.02
0.50	0.05
0.80	0.08
1.00	0.10
1.50	0.15
2.00	0.20
3.00	0.30
4.00	0.40
5.00	0.50

  - Fill each flask to volume with deionized water. These have an expiration life of one year.

- Standard Working 0.01% n-Propanol Solution:
  - Pipette 10 ml of the 1% Stock n-Propanol Solution into a 1000 ml volumetric flask and fill to volume with deionized water to give about a 0.01% working solution. Larger volumes may be prepared as needed. This solution has an expiration life of one year.

  OR
  - Pipette about 124 µL of reagent grade n-Propanol into a 1000 ml volumetric flask and fill to volume with deionized water to give about a 0.01% stock solution. This solution has an expiration life of one year.
- Negative Controls will consist of a mixture of DI water and internal standard (n-propanol).
- Commercially prepared ethanol standards of various concentrations as needed as well as volatile mixtures (also known as resolution standards) are purchased through Cerilliant, Restek or other authorized vendors. These solutions are used both as standards and as positive quality controls.

7 INSTRUMENTATION

Instrument #1: Shimadzu GC 2010 plus Gas Chromatograph equipped with two flame ionization detectors, utilizing the "Class-VP" integration software and two capillary columns such as RTX BAC1 and RTX BAC2 (0.53 mm inner diameter x 30 meters) along with a Teledyne Tekmar HT3 Headspace analyzer. Both columns are attached to a single injection port.

Instrument #2: Agilent 7890A Gas Chromatograph equipped with two flame ionization detectors, utilizing the "OpenLab CDS EZChrome" integration software and two capillary columns such as RTX BAC1 and RTX BAC2 (0.53 mm inner diameter x 30 meters) along with an Agilent 7697A Headspace analyzer. Both columns are attached to a single injection port.

General Requirements for Analytical Instrumentation

- All instruments will be maintained in proper working condition.
- All instruments will be checked after being moved or if a major repair is performed.
- If an instrument fails standardization, performance verification check or a performance problem is detected during routine maintenance, it must be removed from service. The supervisor must be notified and the problem recorded.
- No instrument is to be used if it is not in proper working order.
- Repair or have the instrument repaired and perform routine quality control procedures to ensure it is working properly before the instrument is returned to service.

- The Technical Lead will determine if the instrument is ready to return to service for routine casework.
- A record of all repairs and maintenance will be kept in a maintenance log with the instrument.

Pipettes and dispensers

Pipettes and dispensers in the section will be calibrated externally or replaced at least annually. New or newly calibrated pipettes and dispensers will be evaluated before use. Evaluation will also be conducted after any repairs before being placed back into service. Procedures may include gravimetric or photometric methods. The evaluation will be conducted at the settings called for in the procedure section (section 8).

Gravimetric Method

Allow the temperature of a container of deionized water to equilibrate at least overnight.
Record the temperature (^{0}C).
Record the weight of ten aliquots from each setting to four decimal places.
Convert each weight to volume.

- For the pipettes, convert to microliters:
  - Multiply the weight in grams by 1000. Then divide by the density at the recorded temperature. Refer to the "Density Table".
  - Truncate the calculated volumes to one decimal place and report.
  - Calculate and record the accuracy (the average volume minus the selected volume) at 200 µL.
  - The accuracy must be within ISO 8655 limits of ± 6 µL.
  - Calculate and record the precision (standard deviation) at 200 µL.
  - The precision of each setting must be within ISO 8655 limits of ± 2 µL.
- For the Bottle Top Dispensers, convert to milliliters:
  - Divide the weight in grams by the density at the recorded temperature. Refer to the "Density Table".
  - Truncate the calculated volumes to three decimal places and report.
  - Calculate and record the accuracy (the average volume minus the selected volume) at 2 mL.
    - The accuracy must be within ISO 8655 limits of ± 0.030 mL.
  - Calculate and record the precision (standard deviation) at 2 mL.
    - The precision must be within ISO 8655 limits of ± 0.010 mL.

If the pipette does not meet requirements it may be serviced or repaired by qualified laboratory personnel and rechecked or submitted to a repair company and rechecked or it may be permanently removed from service.

Records

All recorded and reported results above will be kept in the "Pipette Verification" logbook.

Density Table (Handbook of Chemistry & Physics, 62nd Ed.):

Temp (^{0}C)		Density (g/mL)
From	To	
18.0	18.5	0.999
18.6	23.1	0.998
23.2	27.0	0.997
27.1	30.4	0.996
30.5	30.9	0.995

Photometric Method

Photometric evaluations will be conducted according to the manufacturer's operation manual. Manufacturer's specifications must be able to meet ISO 8655 guidelines.

8 PROCEDURE

Evidence Examination

- If there is a valid evidentiary breath test, no further analysis is necessary at this time.
- Examine the evidence and document any discrepancies or irregularities.
- Mark at least the innermost specimen container(s) with the laboratory case number and analyst's initials.
- Mark all tubes with the case number.
  - If more than one tube, mark the tube with the least amount of sample with the item number and use this tube for analysis as long as there is sufficient sample.
- Record characteristics of the tube(s), specimen (i.e. clotted, liquid, etc.) and estimated amounts in the "Item Description" box in LIMS.
- Following sample preparation and analysis, the evidence will be repackaged in the original container and be returned to the Evidence Control Section as soon as is practical.

Sample Preparation

- Allow specimens, standards, and reagents to equilibrate to room temperature.
- Complete a list of sample names and vial locations for the batch.
- Label analysis vials.
- All samples and controls must be prepared with the same reagents.
- Add the following to each analysis vial:
  - A level 1/8th teaspoon of sodium chloride.
  - Two (2) milliliters of the n-Propanol Internal Standard from the dispenser.
  - 200 microliters of the appropriate standard, control or sample with the positive displacement pipetter. Visually inspect the sample container and the analysis vial to insure that each analysis vial is receiving the correct sample.
- Vortex the blood tubes for at least 10 seconds prior to sampling to insure homogenization.
- If a majority of the sample is clotted, the entire sample must be ground to a homogeneous liquid before sampling and analyzed as whole blood by using the tissue grinder.
- For blood tubes with the blood serum separated, the fluid in the serum excluding blood cells may be sampled. These must be converted to whole blood before reporting.
- A new tip and plunger for the micro-pipetter must be used for each standard, control or sample.
- Seal the sample by crimping an aluminum crimp cap and septum onto the top of the vial.

9 ANALYSIS OF THE BLOOD ALCOHOL BATCH

- Each batch will include a set of standards as well as quality control samples.
- The Standard Curve will consist of at least 4 concentration levels and will span from 0.010 to at least 0.400 g/dL. The suggested concentrations are 0.010, 0.100, 0.200, 0.400 g/dL.
- The Standard Curve will be checked for reliability with at least three quality control samples of differing concentrations than those of the Standard Curve. The suggested concentrations are 0.080, 0.150 and 0.300.
- Each batch will include a mixture of common blood volatiles (resolution standard) and a negative control.
- Sample order and position numbers will vary according to the capacity of the headspace unit.
- All samples, standards and quality controls must be analyzed at least in duplicate.
- An air blank must be run between a standard or control and any case sample.

Sample Setup

- Place the analysis vials on the Headspace Autosampler's tray. Insure that they are in the proper order from the list of sample names and vial locations.
- Set up the sequence file in the chromatography software. Insure that they are in the proper order from the list in the previous step.
- Set up the sequence file in the Headspace operating software.
- Start the sequence for the Gas Chromatograph and insure that it is running properly.
- Start the sequence for the Headspace Autosampler and insure that it is running properly.
- Perform the Gas Chromatography analysis on both columns.

10 ACCEPTANCE CRITERIA

New Standards

- Each new lot of commercial or prepared standard or control must be verified by analyzing at least 3 duplicate samples. The duplicates can be all run in one batch or in several batches. All data points must be within ± 5.0% of the mean value and the mean value must be within ± 5.0% of the stated value of each respective standard or control that is 0.100 or greater or within ± 0.010 of the mean value and the mean value must be within ± 0.010 stated value of each respective standard or control that is less than 0.100. Failure of any of these criteria will result in said lot's disqualification from service.
- The Internal Standard and negative control will have an n-Propanol peak that is clearly visible, will be free of all other components and will exhibit a retention time (RT) that is within ± 1% of the expected time for each column.
  - Calculated as follows: $(RT_{(std)} - RT_{(sam)}) \times 100 / RT_{(std)} = \pm 1\%$.
- Failure of any of these criteria will result in said solution's disqualification from service.
- The volatile mixture solution must demonstrate separation between each component by more than 0.10 minutes of the previous compound for each column and will be free of all other components. Failure of any of these criteria will result in said lot's disqualification from service.

New Batch

A batch is considered acceptable when all of the following criteria are met:

- The standards and controls must be within ± 5% of the mean value and mean value must be with ± 5.0% of the stated value of each respective standard or control that is

0.100 or greater or within ± 0.010 of the mean value and the mean value must be within ± 0.010 stated value of each respective standard or control that is less than 0.100.

- The retention times of the standards and controls must be within ± 1% of the expected values for each column.
- The volatile mixture solution must demonstrate separation between each component by more than 0.10 minutes of the previous compound for each column.
- The negative control will have an n-Propanol peak that is clearly visible, will be free of all other components and will exhibit a retention time that is within ± 1% of the expected time for each column.
- Ethanol values of any negative control and any air blank must be below the detection limit of 0.001 g/dL.
- All batch criteria will be documented and included in each case file in LIMS.
- The standard line must have a correlation coefficient (r^2) no less than 0.995.
- Failure of one or more of the above will require the analyst to disqualify the batch and to re-evaluate the system. Any corrective actions will be noted on the Batch Worksheet. Any samples run in the batch will be re-analyzed in a new batch.

Case Samples

- All values must be within ± 5% of the mean value that is 0.100 or greater or within ± 0.010 of the mean value that is less than 0.100.
- The retention times must fall between ± 1% of the expected time for each column.
- Each sample's criteria will be documented and included in each case file in LIMS.
- Failures of these requirements do not fail the run, but only the sample affected. Corrective actions will be noted in the analyst's case notes. The sample will be re-analyzed in a new batch.

11 ESTIMATING UNCERTAINTY (ASCLD 5.4.6)

Ethanol concentrations have been determined to be critical measurement. As such, the measurement uncertainty associated the ethanol concentration must be determined and documented. Below are listed the sources of uncertainty associated with the quantitation of ethanol in blood that have been considered for this method.

- Method Repeatability
  - Method repeatability uncertainty will be determined based on historical data for the controls analyzed with each run. The mean standard deviation from the quality control samples (0.05 g/dL and above) will be used as the uncertainty associated with method repeatability. Method repeatability uncertainty is type A data and has a normal distribution.
- Sample Volume

  - Sample volume uncertainty will be determined based on historical performance data for the pipetter used to deliver the 200 µL of sample. The standard deviation in precision from the annual verification check will be used as the uncertainty associated with sample volume and is type A data. If the pipetter has been calibrated externally, the uncertainty value from the calibration company may be used and is type B data. Sample volume uncertainty has a normal distribution.
- Standard Volume
  - Standard volume uncertainty will be determined based on historical performance data for the pipetter used to deliver the 200 µL of standards and controls. The standard deviation in precision from the annual verification check will be used as the uncertainty associated with sample volume and is type A data. If the pipetter has been calibrated externally, the uncertainty value from the calibration company may be used and is type B data. Sample volume uncertainty has a normal distribution.
- Internal Standard Volume
  - Internal standard volume uncertainty will be determined based on historical performance data for the dispenser used to deliver the 2 ml of internal standard. The standard deviation in precision from the annual verification check will be used as the uncertainty associated with sample volume and is type A data. If the dispenser has been calibrated externally, the uncertainty value from the calibration company may be used and is type B data. Sample volume uncertainty has a normal distribution.

- Standards Concentration
  - Certificates of analysis from the standards manufacturer will be consulted to determine the highest relative percent deviation associated with any particular standard used. Standard concentration uncertainty is type B data and has a normal distribution. The value used will be the uncertainty value divided by the coverage factor reported in the certificate.
- Calculation and documentation of measurement uncertainty
  - Calculate the standard uncertainty based on the type and distribution the data represents.
  - Calculate the relative contribution: determine to what extent the factor affects the overall uncertainty budget. An item that contributes less than 1/3 of the greatest relative contributor is considered to be negligible.
  - Calculate the Standard Uncertainty: $(\sum(u_n)^2)$
  - Calculate the Combined Uncertainty: $U_c = ((\sum(u_n)^2))^{1/2}$
  - Calculate Expanded Combined Uncertainty using the desired coverage factor.
    - The Forensic Chemistry Section will use a confidence interval of 99.7% or a coverage factor of (k=3).

Example Uncertainty Budget Sheet				
Factor	Value (x)	Standard Uncertainty (u), %	Distribution	Relative contribution to u in %
Repeatability (Type A) (Average values for Quality Control samples – 0.080 and greater)	Determined in house annually.	x/1	Normal	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2 /(\sum(u_n)^2$
Volume of sample 200 µL pipetter (Type A or B)	From annual check or calibration	x/1 or 2	Normal	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2 /(\sum(u_n)^2$
Volume of Standard 200 µL pipetter (Type A or B)	From annual check or calibration	x/1 or 2	Normal	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2 /(\sum(u_n)^2$
Volume of Internal Standard 2 mL pipetter (Type A or B)	From annual check or calibration	x/1 or 2	Normal	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2 /(\sum(u_n)^2$
Standards Certificate of Analysis reports (Type B)	Determined by the largest relative % SD	x/2	Normal	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(un)^2 /(\sum(un)^2$
Subtotal of the uncertainty $(\sum(u_n)^2)$		Sum of the square of each of the uncertainty factors		
Uc = square root of $(\sum(u_n)^2)$	Square root of the sum of the squared uncertainty components	%		
Expanded Uncertainty (U); where (k) = 3	Uc times the coverage factor $U = (u_n x 3)$	%		

12 DOCUMENTATION (ASCLD 5.10.2)

The following will be attached to each case from the batch in LIMS:

- A printout of the standard curve.
- All chromatograms generated from all standards and controls.
- All lot numbers of standards and controls used.
- All chromatograms from the case samples.
- The list of sample names and vial locations from this batch.
- All methods, instrument parameters, sequence parameters.
- All quality assurance documentation.
- Any handwritten notes.
- Any emails or other correspondence concerning the case.

13 OUTSIDE TESTING

Samples will be sent to outside laboratories for additional testing if drugs are suspected or in answer to a signed court order.

- The following conditions will apply:
  - If there is a valid evidentiary breath test, no further alcohol testing will be done.
  - Any sample with an alcohol concentration below 0.10 g/dL, will be sent to the appropriate laboratory for drug testing.
  - Any sample with an alcohol concentration above 0.10 g/dL, will only be sent to the appropriate laboratory for drug testing if the evidence is from a case involving a deceased person, sexual assault or other mitigating circumstances as determined by the Technical Lead or Laboratory Supervisor.
- Defense Court Orders
  - If a valid court order requesting the case be analyzed by an independent laboratory is received, the following will apply:
  - If more than one tube is available, take the unopened tube with the most blood and separate it by creating a new item and tag number in Versadex. Link that tag number to LIMS as a new item. Label that item with the new LIMS item number and send that item according to the instructions in the court order.
  - If only one tube was submitted, seal approximately half of the blood, after vortexing, into an appropriate sterile container. Create a new item and tag number in Versadex. Link that tag number to LIMS as a new item. Label that item with the new LIMS case and item number then send it according to the instructions in the court order.

14 PROFICIENCY TESTING (ASCLD 5.9.3)

Proficiency sample will be run as a normal case sample within a batch. They can be run in one batch or across multiple batches.

A proficiency sample is considered within acceptable limits when the following criteria are met:

- All values must be within ± 5% of the mean value that is 0.100 or greater or within ± 0.010 of the mean value that is less than 0.100.
- The retention times must fall between ± 1% of the expected time for each column.
- The "Grand Mean" value reported by the Testing Service or Company must be within The Expanded Uncertainty (U); where (k) = 3 of the reported average.

15 REFERENCES

Books:

"Basic Gas Chromatography" 5th edition, H.M. McNair and E.J. Bonelli, Varian Instruments, 1969

"Toxicology – Mechanisms and Analytical Methods" Vol. II, C.P. Stewart and A. Stolman, Academic Press, 1961, Chapter 4 "Ethyl Alcohol"

"Forensic Science Handbook", edited by R. Saferstein, Prentice-Hall, 1982, Chapter 12 "The determination of alcohol in blood and breath"

"Garriot's Medicolegal Aspects of Alcohol", 5th edition, edited by J.C. Garriot, Lawyers and Judges Publishing Co.

"Biosafety in the Laboratory – Prudent Practices for the Handling and Disposal of Infectious Materials", National Research Council, National Academy Press, 1989

Journal Articles and papers:

T.D. Goldfinger and D. Schaber, "A Comparison of Blood Alcohol Concentration Using Non-Alcohol and Alcohol-Containing Skin Antiseptics", Annuls of Emergency Medicine, Dec 1982, pp. 665/35 – 667/37.

P.V. Taberner, "A Source of Error in Blood Alcohol Analysis", Alcohol & Alcoholism, Vol 24 No. 5, 1989, pp. 489 – 490.

M. Chiarotti and N. De Giovanni, "Acetaldehyde Accumulation During Headspace Gas Chromatographic Determination of Ethanol", Forensic Science International, 20, 1982, pp. 21 – 25.

R.M. Anthony, C.A. Suthelmer and I. Sunshine, "Acetaldehyde, Methanol and Ethanol analysis by Headspace Gas Chromatography", Journal of Analytical Toxicology, Vol 4, Jan/Feb, 1980, pp. 43 – 45.

G.L. Dick and H.M. Stone, "Alcohol Loss Arising From Microbial Contamination of Drivers' Blood Specimens", Forensic Science International, 34, 1987, pp. 17 – 27.

D.M. Penetar, J.F. McNeil, E.T. Ryan and S.E. Lukas, "Comparison Among Plasma, Serum and Whole Blood Ethanol Concentrations: Impact of Storage Conditions and Collection Tubes", Journal of Analytical Toxicology, Vol 32, September 2008, pp. 505 – 510.

D.B. Zittel and G.G. Hardin, "Comparison of Blood Ethanol Concentrations in Samples Simultaneously Collected into Expired and Unexpired Venipuncture Tubes", Journal of Analytical Toxicology, Vol 30, June 2006, pp. 317 – 318.

R.C. Charlebois, M.R. Corbett and J.G. Wigmore, "Comparison of Ethanol Concentrations in Blood, Serum and Blood Cells for Forensic Application", Journal of Analytical Toxicology, Vol 20, May/June 1996, pp. 171 – 178.

C.L. Winek and M. Carfanga, "Comparison of Plasma, Serum and Whole Blood Ethanol Concentrations", Journal of Analytical Toxicology, Vol 11, Nov/Dec 1987, pp. 267 – 268.

M.A. Miller, et al. "Does the clinical use of ethanol-based hand sanitizer elevate blood alcohol levels? A prospective study", American Journal of Emergency Medicine, 24, 2006, pp. 815 – 817.

A.W. Jones, K.A. Jonsson and S. Kechagias, "Effect of high-fat, high-protein and high-carbohydrate meals on the pharmacokinetics of a small dose of ethanol", British Journal of Clinical Pharmacology, 44, 1997, pp. 521 – 526.

C.L. Winek and L.J. Paul, "Effect of Short-Term Storage Conditions on Alcohol Concentrations in Blood from Living Human Subjects", Clinical Chemistry, 29/11, 1983, pp. 1959 – 1960.

D. Yajima, et al. "Ethanol production by Candida albicans in postmortem human blood samples: Effects of blood glucose level and dilution", Forensic Science International, 164, 2006, pp. 116 – 121.

A.W. Jones and K.A. Jonsson, "Food-Induced Lowering of Blood-Ethanol Profiles and Increased Rate of Elimination Immediately After a Meal", Journal of Forensic Sciences, Vol. 39 No. 4, July 1994, pp.1084 – 1093.

D.S. Christmore, R.C. Kelly and L.A. Doshier, "Improved Recovery and Stability of Ethanol in Automated Headspace Analysis", Journal of Forensic Sciences, Vol. 29 No. 4, Oct. 1984, pp. 1038 – 1044.

E.T. Lewis, "Inert Sample Pathway for Blood Alcohols Using a 7000 Headspace Autosampler", Teledyne/Tekmar Instruments, 7000-006.doc; 9-Jun-03.

G.D. Amick and K.H. Habben, "Inhibition of Ethanol Production by Saccharomyces cerevisiae in Human Blood by Sodium Fluoride", Journal of Forensic Sciences, Vol. 42 No. 4, 1997, pp. 690 – 692.

K.M. Dubowski, "Manual for the Analysis of Ethanol in Biological Liquids", Report No. DOT-TSC-NHTSA-76-4-HS-802-208, Jan. 1977.

Perkin-Elmer, "Blood Alcohol Analysis Utilizing Headspace Autosampling and Dual-column GC Confirmation", Field Application Report.

D.A. Labianca, "Conversion of Serum-Alcohol Concentrations to Corresponding Blood-Alcohol Concentrations", Journal of Chemical Education, Vol. 79 No. 7, July 2002, p. 803.

J.G. Wigmore and M. Elliot, "Serum, Blood, and Breath Alcohol Results in a Case of Impaired Driving Causing Bodily Harm", Canadian Society of Forensic Science Journal, Vol. 37 No. 4, 2004, pp. 223 – 227.

K.W. Smalldon and G.A. Brown, "The Stability of Ethanol in Stored Blood Part II. The Mechanism of Ethanol Oxidation", Analytica Chimica Acta, Vol. 66, 1973, pp.285 – 290.

T. Meyer, P.K. Monge and J. Sakshaug, "Storage of Blood Samples Containing Alcohol", Acta pharmacol et toxicol., Vol. 45, 1979, pp. 282 – 286.

H.M. Chen, et al, "Studies of the Oxidation of Ethanol to Acetaldehyde by Oxyhemoglobin Using Fluorigenic High-Performance Liquid Chromatography", Alcoholism; Clinical and Experimental Research, Vol. 18 No. 5, Sept/Oct 1994, pp. 1202 – 1206.

X. Shan, et al, "A study of blood alcohol stability in forensic antemortem blood samples", Forensic Science International , Vol. 211, 2011, pp. 47 – 50.

T. Winek, C.L. Winek and W.W. Wahba, “The effect of storage at various temperatures on blood alcohol concentration”, Forensic Science International, Vol. 78, 1996, pp. 179 – 185.

J. Chang and K. Kollman, “The effect of temperature on the formation of ethanol by candida albicans in blood”, Journal of Forensic Sciences, Vol. 34 No. 1, Jan 1989, pp. 105 – 109.

Courses:

The Indiana University “Robert F. Borkenstein Course On Alcohol and Highway Safety: Testing, Research and Litigation”

“Introduction to Measurement Uncertainty”, parts I, II & III, M.A. LeBeau, Online on demand course through RTI International, 2010.

Websites:

SWGDRUG.org

ASCLD.org

ISO.org