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1 SCOPE

To establish quality assurance guidelines for the analysis of ethyl alcohol in blood specimens.

2 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 material should be thoroughly flushed for at least 15 minutes with cool tap water or at an 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 chemical or biohazard materials and disposal.
- Always use universal precautions (fume or biological hood, gloves, face protection, and lab coat).
- After completion of 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 ensure that no blood is spilled in the work area. It must also be carefully cleaned with a 10% bleach solution or appropriate disinfectant after use.

3 CHEMICALS, REAGENTS AND STANDARDS

- 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
- Sodium Azide – (not less than Reagent Grade)

Standards and Controls

- Each new lot of in-house prepared ethanol standards or controls must be verified by analyzing at least 3 duplicate samples. All the duplicates can be run in one batch or in several batches. The mean value when compared to the stated value of each standard or control must be within the determined uncertainty for the instrument and method used during analysis for any concentration greater than or equal to 0.050 g/dL. For standards and controls less than 0.050 g/dL, the mean value and stated value must be within ±0.003 g/dL. Failure of any of these criteria will result in said lot's disqualification from service.
- Purchased standards and controls from ISO Guide 34:2009 accredited vendors do not require verification. Any purchased materials that are not ISO Guide 34:2009 will require verification as determined by the Technical Leader.
- The Internal Standard and matrix 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: $(RT_{(std)} – RT_{(sam)}) X 100 / RT_{(std)} = ±1\%$.

Failure of any of these criteria will result in said solution's disqualification from service.

Solutions:

- All reagents and prepared solutions will be labeled with the date of preparation, the initials of the preparer, lot number and included in an appropriate logbook. (ASCLD/LAB 5.1.3)
- All reagents and working solutions will be verified before use in casework. (ASCLD/LAB 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.
- 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.

- 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 0.01% n-Propanol Solution:
  - In a volumetric flask, pipette 10 ml of the 1% Stock n-Propanol Solution per 1000 ml of deionized water. Fill to volume with deionized water to give about a 0.01% working solution. Add Sodium Azide (up to 6.5 g per 1000 ml of solution) (Optional). This solution has an expiration life of one year.

  OR
  - In a volumetric flask, pipette about 124 µL of reagent grade n-Propanol per 1000 ml of

deionized water. Fill to volume with deionized water to give about a 0.01% working solution. Add Sodium Azide (up to 6.5 g per 1000 ml of solution) (Optional). This solution has an expiration life of one year.

- 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.
  - The volatile mixture solution must demonstrate separation between each component with retention times that are equal to or greater 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.

4 INSTRUMENTATION AND EQUIPMENT

- Instrument #1: Shimadzu GC2010 Plus Gas Chromatograph (Serial No. C11805250168) equipped with two flame ionization detectors, utilizing the "Lab Solutions" integration software and two different capillary columns capable of exhibiting changes in retention time and change in elution order of common volatiles along with a Shimadzu HS-20 Headspace analyzer (Serial No. O20715200336).
- Instrument #2: Shimadzu GC2010 Plus Gas Chromatograph (Serial No. C11805550372) equipped with two flame ionization detectors, utilizing the "Lab Solutions" integration software and two different capillary columns capable of exhibiting changes in retention time and change in elution order of common volatiles along with a Shimadzu HS-20 Headspace analyzer (Serial No. O20715500620).
- Instrument #3: Shimadzu GC2030 Gas Chromatograph (Serial No. C12255501712) equipped with two flame ionization detectors, utilizing the "Lab Solutions" integration software and two different capillary columns capable of exhibiting changes in retention time and change in elution order of common volatiles along with a Shimadzu HS-20 Headspace analyzer (Serial No. O20715500727).
- Positive displacement micro-pipette with disposable tips and plungers or other pipetting device capable of accurately delivering a 200 microliter sample.
- 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.

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 a performance verification check or a performance problem is detected during routine maintenance, it must be removed from service. The supervisor or technical leader must be notified and the problem recorded.
- No instrument is to be used if it is not in proper working order.
- The instrument must pass a performance verification before the instrument is returned to service.
- The Technical Leader 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.
- Maintenance or service to an instrument is followed by verification that may include analyzing positive, negative, and resolution controls.
- Preventive maintenance is performed as needed by laboratory personnel or a contracted vendor.

General Requirements for 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 prior to being placed back into service. A gravimetric method may be used for evaluation. The evaluation will be conducted at the settings called for in the procedure section.

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 or pipetting devices, 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.
    - Calculate and record the precision (standard deviation) at 200 µL.
        - The precision of each setting must be within ISO 8655 limits.
- 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.
  - Calculate and record the precision (standard deviation) at 2 mL.
    - The precision must be within ISO 8655 limits.

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

5 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 is submitted, mark the tube that will be analyzed with the item number
- Record characteristics of the tube(s), specimen, and estimated amounts 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 pipette. Visually inspect the sample container and the analysis vial to ensure that each analysis vial is receiving the correct sample.
- Vortex the blood tubes for approximately 10 seconds prior to sampling to ensure homogenization.
- If a majority of the sample is clotted, the entire sample may be ground to a homogeneous liquid before sampling and analyzed as whole blood by using the tissue grinder.
- A new tip and plunger for the micro-pipette must be used for each standard, control or sample.
- When using a pipetting/diluting device, steps must be taken to ensure that cross contamination does not occur between aliquots.
- Seal the sample by crimping a crimp cap and septum onto the top of the vial.

6 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 5 concentration levels and may span from 0.010 to at least 0.400 g/dL. The suggested concentrations are 0.020, 0.050, 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 and manufacturer (if available) than those of the Standard Curve. The suggested concentrations are 0.080, 0.150 and 0.300. A quality control sample containing ethanol and whole blood as a matrix may also be used.
- Each batch will include one of each:
  - A mixture of common blood volatiles (volatile mix solution).
  - A reagent control.
  - A negative matrix control.
- All other samples, standards and quality controls must be analyzed at least in duplicate.
- An air blank must be run between a standard or control that precedes any case sample.

Sample Setup

- Place the vials on the Headspace's auto-sampler tray. Ensure that the vials are in the proper order from the list of sample names and vial locations.
- Set up the sequence file in the chromatography and headspace operating software. Ensure that the vials are in the proper order from the list in the previous step.
- Start the sequence for the Gas Chromatograph and Headspace auto-sampler.
- Perform the Gas Chromatography analysis on both columns.

7 ACCEPTANCE CRITERIA

Standard and Control Samples

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

- For standards and controls less than 0.050 g/dL in concentration:
  - The mean value when compared to the stated value must be within ±0.003 g/dL.
  - Each value for that standard or control must be within ±0.003 of the mean value.
- For standards and controls equal to or greater than 0.050 g/dL in concentration:
  - The mean value when compared to the stated value of each standard and control must be within the determined uncertainty for the instrument and method used during analysis. Additionally, each value for that standard or control must be within the determined uncertainty of the mean value.
- "Retention time" is defined as the peak maxima.
- 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 with retention times that are equal to or greater than 0.10 minutes of the previous compound for each column.
- Negative controls 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 established for the respective instrument.
- 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 in the case record. Any samples run in the batch will be re-analyzed in a new batch.

Case Samples

- If the mean value is less than 0.050 g/dL, values must be within ±0.003 g/dL of the mean value. Otherwise, values must be within the determined uncertainty for the instrument and method used during analysis of the mean value.
- The retention times must fall between ± 1% of the expected time for each column.
- If the concentration of a sample is greater than the upper limit of the standard curve, the sample will be diluted one to one with DI water and reanalyzed.
- 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 case record. The sample will be re-analyzed in a new batch.

8 CASE DOCUMENTATION (ASCLD/LAB 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 specific to the case.
- 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.

9 EVIDENCE DISPOSTION

Samples may 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 0.08 g/dL, can be sent to an appropriate laboratory for drug testing at the request of a customer.
  - Any sample with an alcohol concentration above 0.08 g/dL, will be sent to an appropriate laboratory for drug testing at the discretion of the 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 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 the unopened tube contains an insufficient volume of sample for independent analysis, the tube containing a sufficient amount of sample may be sent.
  - If only one tube was submitted, send the item according to the instructions in the court order.

10 ESTIMATING UNCERTAINTY (ASCLD/LAB 5.4.6)

Ethanol concentrations have been determined to be a critical measurement. As such, the measurement uncertainty associated the ethanol concentration must be determined and documented. Listed below are 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 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 Toxicology 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)	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_nx3)	%

11 PROFICIENCY TESTING (ASCLD/LAB 5.9.3)

Each proficiency sample will be run as a normal case sample. The samples may be run in one batch or across multiple batches.
A proficiency sample is considered within acceptable limits when the following criteria are met:

- All samples must meet and pass the same acceptance criteria used for unknown blood samples.
- 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.

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