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REVISION HISTORY

Effective Date	Brief Description of Change(s)
08/22/2019	Original Issue Previous revision history for individual chapters included in archived documents
10/17/2019	Administrative Revision: BA-02-01

01 QUALITY ASSURANCE

BA-01-01 CHEMICALS, REAGENTS, AND STANDARDS

1 Scope

To establish quality assurance guidelines for the use of significant chemicals/reagents and ethanol standards.

2 Related Documents

CLS Manual – Laboratory Equipment

3 Practices

3.1 Chemicals/Reagents

A. All purchased chemicals/reagents at a minimum, must be manufactured for general laboratory use.

B. Prepared reagents must be quality tested prior to its first use with evidentiary items.

1. Acceptance criteria can be found in the respective reagent's preparation instructions.
2. The resulting data will be kept in retrievable form in the laboratory.

C. Quality control of gases used in analysis is documented as a component of the analytical process by acceptable performance of control standards and successful calibration.

D. Purified water is quality tested as a component of the aqueous method blank to verify that it contains no detectable volatiles (n-Propanol is added as internal standard).

3.2 Ethanol Standards

A. Ethanol standards will be purchased from an approved supplier.

B. Before using an ethanol standard, the standard or a sample from the same batch/lot will be analyzed using previously verified laboratory standards to ensure its concentration is correct as labeled or prepared.

1. The resulting data will be kept in retrievable form in the laboratory. The records shall contain all pertinent information including the lot number, source, initials of the chemist who performed the test, whether the results are acceptable, and the date approved for use in casework.
2. The results of the analysis of each ethanol standard at or above 0.080 g/100mL must be within 5% of its expected value.

$$\frac{|Observed - Expected|}{Expected} \times 100$$

Results below 0.080g/100mL must be within 0.004 g/100 mL of its expected value.

C. Ethanol standards shall not be used for casework beyond the manufacturer's expiration date.

BA-01-02 Equipment

1 Scope

To establish quality assurance guidelines for the maintenance, calibration, and repair of analytical equipment. Quality assurance guidelines for significant chemicals and reagents are discussed in BA-01-01, Chemicals, Reagents and Standards.

2 Related Documents

CLS Manual – Laboratory Equipment

CLS Manual – Validations and Performance Verifications

BA-02-04 – Validation of Significant Equipment

3 Maintenance, Calibration and Validation of Significant Equipment

The processes summarized in this section are meant to be supplementary to the policies outlined in the chapters of the CLS Manual regarding *Laboratory Equipment* and *Validations and Performance Verifications*. Laboratory personnel are responsible for consulting the CLS Manual to ensure all policies are followed.

3.1 General Requirements for Analytical Equipment

A. All equipment will be maintained in proper working condition and properly calibrated. No equipment is to be used if it is not in proper working order.

B. New models of significant equipment will be validated according to the Validations and Performance Verifications chapter of the CLS Manual.

C. Validations and/or performance verifications of new significant equipment will be recorded on the LAB-408.

D. Maintenance activities performed on significant equipment will be recorded on the LAB-405.

E. Performance Checks

1. A performance check is required for the following:

   a) Equipment has been moved

   b) Routine/preventative maintenance

   c) Minor repairs – a repair that did not alter the ability of equipment to perform as expected

2. Performance check results are recorded on the LAB-405.

F. Out of Service Events

1. Equipment is taken out of service for the following:

   a) Does not pass a performance check after troubleshooting and correction

   b) Gives questionable results

   c) Non-functional

2. The equipment is isolated to prevent its use or clearly labelled or marked as out of service, and the LAB-405 is updated.

3. The incident is recorded on the LAB-410 and given to the Section Supervisor, Technical Leader, and/or TPOC. A Quality Incident will be initiated.
4. To return the equipment to service, follow the process outlined in the Performance Verifications section.

G. Performance Verifications

1. A performance verification is required for the following:
   a) *Equipment is being returned to service following an out of service event*
   b) *Major repairs*
   c) *Equipment went off-site for repair, calibration, maintenance or modification*
2. The activity and performance verification results are recorded as follows:
   a) *LAB-405 – Approval needed from the Section Supervisor, Technical Leader, and/or TPOC*
   b) *LAB-408 – Approval needed from local laboratory*

   Technical review required by Advisory Board Chair and either Team Leader or TPOC, only when returning significant equipment to service.

3.2 Gas Chromatograph

A. New gas chromatograph models will be validated according to the Validations and Performance Verifications chapter of the CLS Manual.

B. Specific requirements for the validation of a new gas chromatograph are outlined in BA-02-04.

C. If equipment repairs are performed that may have an effect on the lab's established LOD (e.g. replacement of a detector), the LOD determination will be re-confirmed via performance verification.

D. If equipment repairs are performed that could affect retention times (e.g. installation of new gas chromatograph columns), the retention time of each of the following volatiles will be verified individually as a performance check.

1. Ethanol
2. Acetaldehyde
3. Formaldehyde
4. Methanol
5. Isopropanol
6. Acetone
7. Toluene (optional)
8. n-Propanol

3.3 Balances

A. New balance models will be validated according to the Validations and Performance Verifications chapter of the CLS Manual.

B. Balances shall be calibrated and certified by an external vendor once a year. Laboratory calibration weights should be checked after the annual re-certification of the balance.

C. Keep the balances clean and leveled at all times.

D. A performance check is required at least quarterly on all balances that are in service.

1. Balance accuracy will be verified using standard weights. The values at which the balances must be checked include (where the balance is capable):

   a) Analytical balances: 10mg, 100mg, 1g, 10g

   b) Top loader balances: 1g, 4g, 10g, 100g, 200g, 400g

2. Since the tolerances of electronic balances may vary, the specific equipment specifications must be checked to determine the appropriate criteria for satisfactory performance. Each lab shall establish performance criteria specific to their balances.

3. In addition to documenting the activity on the LAB-405, maintain a log with the results of any performance checks.

3.4 Diluter/Dispenser and Mechanical Pipettes

A. New diluter/dispenser and mechanical pipette models will be validated according to the Validations and Performance Verifications chapter of the CLS Manual.

B. New mechanical pipettes must be verified before initial use in casework. The pipette must meet the manufacturer's specification for accuracy and the coefficient of variation must be within the manufacturer’s specification or 1%, whichever is greater.

C. Specific requirements for the validation of a new automated diluter/dispenser are outlined in BA-02-04.

D. Diluter/dispensers and mechanical pipettes shall be calibrated and certified by an external vendor once a year.

E. A performance check is required at least twice a year on all diluter/dispensers and mechanical pipettes that are in service.

1. The gravimetric method will be used to collect four (4) or more data points for each pipette/syringe independently to verify delivery volume(s) relevant to casework.

2. Data points must meet the manufacturer’s specification for accuracy and the coefficient of variation must be within the manufacturer’s specification or 1%, whichever is greater.

3. Performance check may be conducted by personnel within the laboratory or an approved external service provider.

4. In addition to documenting the activity on the LAB-405, maintain a log with the results of any performance checks.

4 General Equipment

4.1 Refrigerators

A. Refrigerators used in the laboratory should be capable of maintaining the optimum temperature range of 2-8°C for storing reagents and samples.

1. If the temperature is not being maintained at the prescribed values, verify that the temperature regulation control is at the proper setting and adjust if necessary.

2. The temperature will be rechecked to ensure the temperature is within the acceptable range. It may also be necessary to reduce over-crowding in an effort to increase air circulation in all compartments.
3. If necessary, a technical representative may need to be called for service or the refrigerator may need to be replaced if corrective action is not adequate.

B. The temperature will be monitored and recorded at least weekly.

4.2 Thermometers

Thermometers used to monitor refrigerator temperatures will either be NIST traceable, or be verified with a NIST traceable thermometer annually at a temperature within the optimum range to ensure that the temperature being measured is within 1.0°C of the NIST traceable thermometer's reading.

A. If the thermometer varies greater than 1.0°C, then the amount of deviation will be noted.

B. If the deviation of the thermometer is greater than 2.0°C, then the thermometer must be replaced.

5 Records

LAB-405 – Equipment Log

LAB-408 – Validation/Verification Form

LAB-410 – Equipment Out of Service Incident Form

BA-01-03 STORAGE AND DISPOSITION OF TOXICOLOGICAL EVIDENCE

1 Scope

The following guidelines pertain to the storage, transfer, and/or disposition of toxicological evidence.

2 Related Documents

CLS Manual – Evidence and Database Sample Integrity

CLS Manual – Return of Evidence

TOX-02-02 – Disposition of Toxicological Evidence

3 Practices

3.1 Storage of Toxicological Evidence

All liquid whole blood specimens and any other liquid body fluids are stored refrigerated prior to and post analysis.

3.2 Disposition of Toxicological Evidence

A. Non-DPS evidence will be returned to the submitting agency.

B. DPS evidence will be retained until the court-authorized disposition date.

C. Evidence to be forwarded to or analyzed in the Austin Crime Laboratory

1. Evidence other than blood or urine (e.g. vitreous)
2. Evidence received for drug analysis only
3. Evidence related to sexual assault cases
4. Evidence in which both alcohol/volatiles and drug analysis is requested
   - *a) Analyze the evidence for alcohol content.*
   - *b) Forward specimens with an alcohol content of less than 0.100 g/100 mL.*
   - *c) If the alcohol content is determined to be equal to or greater than 0.100 g/100 mL (including an equivalent breath alcohol test result), drug analysis will not be routinely performed unless it is an analysis of a specimen from a living driver in an incident involving a deceased victim or it is a non-traffic offense (e.g., death investigation or drug facilitated sexual assault).*
     - *i. Considerations may be made with a written request from the prosecuting agency.*
     - *ii. No verbal requests should be considered.*
5. The originating laboratory's report will indicate that the specimen was forwarded to the Austin Crime Laboratory for drug screening.

4 Literature References and Supporting Documentation

Ballou, S, Stolorow M, Taylor M Bamberger P.S. Brown L Borwn R...Stoiloff S (2013 April). "The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers". National Institute of Standards and Technology. National Institute of Justice. doi:10.6028/NIST.IR.7928

BA-01-04 CASE DOCUMENTATION

1 Scope

These policies are established as minimum requirements for case documentation and recordkeeping for Toxicology (Alcohol/Volatiles) cases.

2 Related Documents

CLS Manual – Laboratory Records

CLS Manual – Electronic Storage and Archival of Records

CLS Manual – Records Requests and Release of Laboratory Records and Information

3 Practices

3.1 Contents of Case Records

A. Laboratory Report

B. Toxicology Request Submission Form (LAB-203) or Laboratory Submission Form (LAB-201)

C. Toxicology (Alcohol/Volatiles) Analysis Worksheet (LAB-BA-01) or electronic equivalent

D. Administrative documentation

E. Analytical data

1. Chromatograms in the case will be maintained and archived in the LIMS. Each page will include case number, handwritten or electronic initials of analyst, and date of testing. Either the batch record or a reference to the relevant batch record will be maintained in the case record.

2. The analyst's review of the data is concluded on the date of the original Draft Complete milestone in the LIMS. If the analyst must edit the record after that date, their original review date will be recorded in the case record.

3.2 Batch Records

A. Each batch of samples will include an archive of data relevant to the batch.

1. Record includes:

   a) Analysis sequence (Order in which samples and standards are analyzed, listed with case number)

   b) Subject name (optional)

   c) Analyst initials or equivalent identifier

   d) Results

   e) Standard lot numbers

   f) Batch date / ID

   g) Calibration report

   h) Instrument parameters

   i) Any additional items required by the Laboratory Records chapter of the CLS Manual.

2. Chromatograms of standards and method blanks

B. The batch records will be maintained in the laboratory, however if maintained electronically, must be archived in LIMS or in a secure alternative electronic location.

3.3 Control Plots

The control plots and summaries for calibration checks and controls will be maintained and archived.

3.4 Batch Raw Data

The chromatograms contained in the case record are the graphical representation of the data. Although batch raw data is not part of the case record, it will be subject to records requests if it is available.

BA-01-05 APPROVED STANDARD ABBREVIATIONS

1 Scope

This is a listing of abbreviations commonly used in the Toxicology (Alcohol/Volatiles) examination documentation. This list has been generated to assist in the interpretation of case file notes and is not a standardized list of required abbreviations.

Note that abbreviations may appear as uppercased or lowercased in case records. Abbreviations may be made plural with the addition of “s” or “ ’s”

2 Abbreviations

Agi	Agitate
APC	Advanced Pressure Controller
BCR	Blood Collection Report
BK	Blood Kit
BL, Bld	Blood
Blk	Black
BSB	Biological Specimen Box
BT	Blood Tube
Cal Check	Calibration Check
Cer	Cerilliant
Conc	Concentration
CTRL	Control
Cyl, CY	Plastic cylinder/tube as used in blood kit
Del	Delay
Det	Detected
Dil	Dilution, Diluted
Dk	Dark
DNR	Do Not Report
Enc	Enclosed
Env	Envelope
Equilib	Equilibrating
FID	Flame Ionization Detector
FN	First Name
GC	Gas Chromatography
Grn	Green
GT	Gray Top Tube
HS	Headspace
ID	Inner Dimension

Incubat	Incubation
Ind	Indications
Inj	Injection
IP	Inner Packaging
ISTD, IS	Internal Standard
LN	Last Name
LOD	Limit of Detection
LOQ	Limit of Quantitation
LV	Low Volume
MI	Middle Initial
MN	Middle Name
ND	Not Detected
Neg	Negative
n-prop	n-Propanol
OP	Outer Packaging
PB	Plastic Box
POR	Police Officer Report
Pst	Post
PT	Purple Top Tube
PW	Paperwork
Res	Restek
Ret Time, RT	Retention Time
RL	Red Label
SC	Specimen Container
SCF	Signed Consent Form
STD	Standard
Syr	Syringe
T. Dbl	Time when peak width doubles
Temp	Temperature
UCF	Unsigned Consent Form
UR	Urine
YE	Yellow Envelope
Zip	Ziplock

BA-01-06 GLOSSARY OF TERMS

1 Scope

The following terms are used in the analysis of biological samples for ethanol and other volatiles.

2 Definitions

Aliquot: The portion of the sample that is used for analysis.

Analysis Session: The set of samples that are subjected to the same calibration and conditions. More than one analysis session can be related to a single set of cases.

Area Ratio: Target analyte area counts divided by internal standard area counts, used in the calculation of the target analyte concentration. This is also referred to as the Response Ratio.

Batch: The set of cases and standards analyzed by a single analyst in one or more analysis session(s).

Calibration Check Standard: A check on the stability and validity of a calibration.

Calibration Curve: A plot of instrument response versus concentration generated by the analysis of multiple calibrators.

Calibrator: A solution of known concentration that is used to establish the relationship between what is measured (the response) and the analyte concentration.

Control: Any sample used to verify the performance of the instrument. This includes blanks and the volatile mixture.

- **Negative Control:** Specimen containing no analyte except internal standard (when prepared), used to verify the absence of interference. (e.g. blanks, blood blanks).
- **Positive Control:** Specimen containing analyte at a known concentration and/or identity (e.g. purchased standards, volatile mix).

Cut-off: An administratively defined response or concentration that is used to discriminate between positive and negative results. Analytes observed below cutoff are considered “not detected”. *See also* Limit of Detection

Internal Standard: A compound, structurally related to the analyte being measured, that is needed for accurate quantitation. The internal standard goes through the analysis process in a manner similar to the analyte of interest.

Limit of Detection: An estimate of the lowest concentration of an analyte in a sample that can be reliably detected or identified, but not necessarily quantitated, by the analytical method.
Note: The limit of detection for ethanol is administratively defined in the SOP.

Limit of Quantitation: An estimate of the lowest concentration of an analyte in a sample that can be reliably measured with acceptable bias and precision.
Note: The limit of quantitation for ethanol is administratively defined in the SOP.

Sequence: The order in which a set of cases and/or standards are analyzed on an instrument.

Specimen Container: A cup, tube, or other receptacle containing a biological fluid.

Standard: Reference materials used to verify the performance of a method, procedure, instrument, or reagent. This includes calibrators, calibration checks, and controls.

Tolerance: The allowable difference between the calculated concentrations of the two aliquots of an unknown sample.

Note: The tolerance has been administratively defined in the SOP.

Volatiles Mixture: A laboratory prepared or purchased solution of various volatile compounds that can be found in the human body, either endogenously or by other means of introduction into the body, e.g. ingestion, inhalation, or absorption through the skin.

Within: Not beyond the limits of; not differing by more than (a specified amount).
Note: this includes the outer bounds of a specified range, e.g. 0.105 g/100 mL is within 5% of 0.100 g/100 mL.

3 Literature References and Supporting Documentation

Indiana University – The Robert F. Borkenstein Course on Alcohol and Highway Safety: Testing, Research, and Litigation, "Glossary of Terms", compiled by A.W. Jones, B.Sc., Ph.D., D.Sc.

"Standard Practices for Method Validation in Forensic Toxicology", Scientific Working Group for Forensic Toxicology (SWGTOX), May 2013.

Levine, Barry, ed. Principles of Forensic Toxicology. 4th ed. Revised. Washington, D.C.: American Association of Clinical Chemistry. 2013.

"Mandatory Guidelines for Federal Workplace Drug Testing Programs", Federal Register vol. 82, No. 13, January 2017

BA-01-07 GUIDELINES FOR CASE REVIEW

1 Scope

The technical and administrative reviews are documented reviews of the entire case record. All relevant documents will be reviewed prior to the release of results. The technical review shall be conducted by an authorized individual.

2 Related Documents

BA-01-04 – Case Documentation

BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis

CLS Manual – Laboratory Records

CLS Manual – Laboratory Reports, Letters, and Certificates

CLS Manual – Review of Laboratory Records

3 Practices

3.1 Items Examined During a Case Review

A. Case Record

Ensure that all required documents are present and documents generated by an examiner are labeled with the case number, handwritten or electronic initials of the examiner, and the date generated.

B. Batch Record

1. Ensure that all required documents have been included in the case record or properly archived.
2. Verify that the pipette and/or diluter/dispenser has been recorded.
3. Verify that the instrument ID has been recorded.
4. Check Equipment Log(s) to ensure in service status.
5. Ensure that all agreements meet the required specifications. This includes the agreements for standards and samples.
6. Check calibration results and ensure that the coefficient of determination is greater than or equal to 0.9950.
7. Verify that the Volatile Mix and Internal Standard preparation dates have been recorded.
8. Examine the chromatograms for all standards to ensure that the baseline is steady and all expected peaks are present.
9. Examine the chromatograms for all blanks to ensure that the baseline is steady and there are no unexpected peaks present.
10. Examine area counts of both ethanol and n-propanol for consistency.

C. Worksheet

1. Verify that the specimen type has been selected.

2. Verify that the following items are properly recorded:
   a) *Name on specimen*
   b) *Location of subject name*
   c) *Location of innermost seal*
   d) *Condition of sample*
   e) *Volume of specimen*
   f) *Other notes as needed*
3. If multiple exhibits were submitted, ensure the appropriate exhibit was selected for analysis.
4. If applicable, ensure the correct units are listed and that the units correlate to the selected specimen type.
5. Ensure results match the sample chromatograms, batch record and report.

D. Sample Chromatograms
1. Examine to ensure that the baseline remains steady.
2. Examine area counts of both ethanol and n-propanol for consistency.
3. Ensure results match the worksheet, batch record and report.
4. If additional volatiles are detected on both columns ensure that the examiner included any that are required on the report.

E. Report
1. Verify that all administrative information matches the submission form.
2. Ensure that the request was properly entered.
3. Ensure results match the worksheet, sample chromatograms, and batch record.
4. Verify the current uncertainty is being displayed as required.
5. If required, verify that the proper disposition note has been included.

F. Other
1. Verify the chain of custody.
2. If needed, verify that a Toxicology (Drugs) analysis request was generated.

3.2 Additional Items if Standards/Samples were Retested

A. Verify that the explanation for retesting has been included in the case record.

B. When using the Shimadzu reporting software, an additional page will generate at the beginning of the report when a standard or sample does not meet the required agreement tolerance. If this occurs, verify that the reason/note provided on this page properly describes what occurred.

C. Ensure data from all sets of analysis have been included in the case record.

D. Verify that the proper set of data was processed and reported. This can be done by viewing and/or reprocessing the raw data or by verifying that the area counts are unique to each analysis session.

E. Compare and evaluate results from each analysis session to ensure they are reasonable.

4 Records

The technical and administrative reviews will be documented in the LIMS.

02 INSTRUMENTAL ANALYSIS

BA-02-01 TOXICOLOGY (ALCOHOL/VOLATILES) ANALYSIS

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 Related Documents

BA-01-01 – Chemicals, Reagents and Standards

BA-02-03 – Instructions for using the Hamilton Microlab

BA-03-01 – Preparation of NaCl/n-Propanol Internal Standard Solution

BA-03-02 – Preparation of Volatile Mixture Standard

Safety Manual – Biological Pathogen Exposure Control Plan

LAB-SAF-02 – Hepatitis B Vaccination Declination Waiver

3 Safety

A. Universal bloodborne 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 required.

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

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

4 Equipment and Materials

- Gas Chromatograph with Flame Ionization Detector, data collection and reporting system
- Two different chromatographic columns suitable for toxicology (alcohol/volatiles) analysis
  - Quantitative column (Restek BAC-1 or comparable)
  - Qualitative column (Restek BAC-2 or comparable)
- Headspace Autosampler (optional)
- Crimper
- Vials, caps, and stoppers
- Positive displacement or air displacement micropipette and tips, or automatic diluter/dispenser
- Ground-glass tissue grinder
- Purified water
- Ethanol standard solutions (NIST Traceable)
  - 0.080 g/100 mL Ethanol Standard
  - 0.150 g/100 mL Ethanol Standard
  - 0.010 g/100 mL Ethanol Standard
  - 0.050 g/100 mL Ethanol Standard

- 0.100 g/100 mL Ethanol Standard
- 0.300 g/100 mL Ethanol Standard
- 0.500 g/100 mL Ethanol Standard

- NaCl/n-Propanol internal standard solution
- Volatile mixture standard

5 Standards, Controls, and Calibration

5.1 Calibration

A. The system must be calibrated for each analysis session of samples examined.

B. Calibrator series: 0.010, 0.050, 0.100, 0.300, and 0.500 g/100 mL ethanol standards

C. Calibration curve criteria for quantitative column:

1. Linear function with inverse weighting and not forced through the origin
2. The coefficient of determination (R^2) will be greater than or equal to 0.9950 to establish linearity

5.2 Ethanol Solutions

A. Calibration checks will include at least the 0.010, 0.100, and 0.500 g/100 mL ethanol standards. They must be separate aliquots from the calibrators.

B. Control samples will include at least one 0.080 and one 0.150 g/100 mL ethanol standard.

C. The results of the analysis of each ethanol standard at or above 0.080 g/100mL must be within 5% of its expected value. The variation will be calculated using the following equation.

$$\frac{|Observed - Expected|}{Expected} \times 100$$

Results below 0.080g/100mL must be within 0.004 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 issue will be corrected, documented and reanalysis of standards will be performed.
2. 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. The issue will be documented.
3. If the analyst determines that the failure of a quality control may be an isolated incident, it will be documented and may be re-analyzed at the end of the analysis session.

D. The laboratory will maintain control plots of results of calibration checks and controls in batches for detection of trends such as calibrator or control decline.

1. Results from at least one batch per week will be plotted. If the laboratory has not performed a batch within that time frame, the next batch performed will be plotted.
2. Each standard lot number will have a separate plot.
3. Control plot summary should include for each data point: batch date, measured concentration, and % difference from expected value.

4. Control plot data summary should include a cumulative average, standard deviation, and % relative standard deviation.

5. Control plot trends will be reviewed by the technical point of contact (or designee) at least annually and documentation will be maintained with the control plot data.

5.3 Non-ethanol Solutions

A. Aqueous or blood matrix method blank – At least one aqueous or blood matrix method blank, which includes n-Propanol internal standard solution, 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 analysis session to demonstrate that ethanol and other volatiles are capable of being separated and identified in a mixture. Compare the retention times to the previously verified retention time for each individual component. Ethanol in the volatile mixture standard is for qualitative purposes only and is not required to meet quantitative criteria.

C. NaCl/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.

6 Procedure

6.1 Evidence Tracking

A. Evidence is assigned a laboratory case number and an evidence exhibit identifier (for cases that have more than one exhibit).

B. Multiple specimen containers within the same parent container of the same type of specimen (and same type of blood tube, if blood), documented as collected within 30 minutes of one another from the same individual are collectively considered to represent a single evidence exhibit.

C. Laboratory case number, subject name, or other unique information are matched to the specimen container when aliquots of sample are drawn.

D. The laboratory case number and evidence exhibit identifier (if applicable) are used in labeling specimen containers during processing.

E. The laboratory case number is used to identify case documents and reports.

F. The laboratory case number is used to match analysis vials to instrument sequence lists.

6.2 Evidence Examination

A. All specimens within the same laboratory case number will be refrigerated except during evidence handling (e.g. evidence examination and sample preparation).

B. For traffic cases, only one toxicology (alcohol/volatiles) analysis will be performed per incident per subject.

1. If there is a complete evidentiary breath alcohol test, no further analysis will routinely be performed, unless other volatiles are suspected (e.g., inhalants).

2. If multiple blood exhibits are submitted, a sample from the exhibit collected closest in time to the incident will be analyzed, unless it is specifically noted/requested by the submitter to analyze a particular exhibit.

3. If a gray top tube is submitted with other types of blood tubes, then only the gray top tube will be analyzed regardless of the collection time (unless specifically noted/requested by the submitter to test a different tube).

4. If both blood and urine are submitted, only the blood will be analyzed.

C. For sexual assault cases, both blood and urine will be analyzed if both are submitted. If the time of collection is greater than 12 hours from the time of the incident, the analyst may choose to analyze only the urine sample

D. For cases affected by a warrantless blood draw, a second analysis will be conducted by court order or with the documented approval of the Laboratory Manager following a customer request.

1. A second tube obtained through an evidentiary search warrant must be submitted.

2. If the second tube is from the same kit, it must be sub-itemized in LIMS.

3. The customer will be informed that the two results will not be used by the analyst to establish an elimination rate. Results between two reports may vary due to a number of factors including, but not limited to:

   a) *Storage conditions (e.g. kit refrigeration between analyses)*

   b) *Length of storage time*

   c) *Length of time between blood draws*

   d) *Difference in preservative type between tubes (e.g. gray versus purple top)*

   e) *Difference in matrix (e.g. serum versus whole blood)*

4. If the initial request included toxicology (drugs) analysis, the Laboratory Manager or designee will communicate with the customer and determine if a second toxicology (drugs) analysis is also needed. If a second toxicology (drugs) analysis is needed, the customer will be advised to contact the toxicology section in Austin to submit the request.

E. Sample Selection and Examination

1. Selection of the specimen container by preservative (color top) should follow the below descending order of preference:

   - *Gray top tubes with sodium fluoride and potassium oxalate preservative*
   - *Purple top tubes with EDTA anticoagulant*
   - *Red top tubes with no preservative added*
   - *Tubes of other color tops for various preservatives*
   - *Serum/Plasma separator tubes*
   - *Other specimen container*

   When there are additional factors to consider (e.g. gap in collection times), the analyst may use their judgment in selecting the appropriate specimen to ensure the specimen tested will produce forensically relevant results. The reasoning will be documented in these circumstances.

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 evidence exhibit number (as applicable). Place the analyst's initials on the sampled specimen container.

   Optionally, if the specimen container's proximal packaging is sealed, the laboratory case number and evidence exhibit number (as applicable) may be placed on the proximal container of any unsampled specimen container(s) to avoid breaking the evidence seal.
4. Record characteristics of the specimen (e.g. condition, estimated volume, 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.

F. Limited Sample

1. If there is less than 2 mL of specimen and both alcohol/volatiles and drug toxicology analysis is requested, the submitting officer may be advised to contact the Austin Crime Laboratory Toxicology Section to discuss the options for testing, prior to conducting alcohol/volatiles analysis. The sample may need to be diluted to perform alcohol/volatiles analysis.
2. If there is less than 1 mL of specimen and the submitting officer still requests both alcohol/volatiles and drug toxicology analysis, a dilution will be performed to conduct alcohol/volatiles analysis.

6.3 Sample Preparation

A. Allow specimens, standards, and reagents to equilibrate to room temperature.

B. Label analysis vials.

C. All samples and controls must be prepared with the same reagents. Each analysis vial shall contain:

1. 0.2 mL of the respective specimen, standard, or reagent.
2. 1.4 mL NaCl/n-Propanol internal standard solution.

D. Considerations when adding an aliquot of the respective specimen, standard, or reagent:

1. Some samples may need the use of a manual pipetting process.
2. If blood specimens are analyzed for alcohol where the specimen exhibits a minimal amount of clotting, invert the tube several times to facilitate mixing.
3. 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.
4. For serum/plasma separator blood tubes with the blood serum/plasma separated, the fluid in the serum/plasma excluding blood cells may be sampled.
5. If urine or vitreous is analyzed, the fluid in the container may be sampled.

6. If limited sample is available for analysis, then with the normal sampling volume (200 µL) add one aliquot of sample to three aliquots of the same source of water used for the aqueous method blank in a small vial to obtain a sufficient dilution for analysis. Mix the sample. Analyze the resulting mixture, adjusting the results for dilution (i.e. multiply by 4).

   Note: With air displacement pipettes, each sample aliquot must be drawn with a new tip. With positive displacement pipettes or automatic diluter/dispenser, the tip must be rinsed thoroughly before pipetting the next sample, unless the pipette is designed to use disposable tips.

   Note: Use of an automatic diluter/dispenser equipped with a reusable tip must be rinsed internally by dispensing at least 1.0 mL internal standard solution and externally with purified water, and wiped with tissue paper or other disposable wipe between samples.

7. Duplicate samples must be prepared either at this time or separately.

8. At least one known ethanol standard will be pipetted prior to the preparation of all specimens and one will be pipetted after the preparation of the last specimen to monitor the consistency of the results.

6.4 Gas Chromatography Analysis

A. Create an analysis sequence for all specimens and standards to be analyzed.

B. Perform Gas Chromatography analysis with two different chromatographic columns.

C. Chromatograms of samples will be retained in the case record. Ethanol and n-Propanol peak identification, retention time, and peak area must be included with the chromatograms. Quantitation results must be included for the quantitative column only and will be recorded to four decimal places. Chromatograms of standards and controls will be kept in a retrievable form in the laboratory.

D. Retain the instrumental parameters in the case file and/or in a retrievable form in the laboratory.

7 Interpretation

7.1 Evaluation of Ethanol Results

A. Specification for Qualitative Results

1. The retention time window in the analysis method must be set to 2% of the established retention time. The detected ethanol peaks in the sample must be within that window.

2. The ethanol peak must be detected on both chromatographic columns to evaluate the quantitative result.

B. Specification for Quantitative Results

1. For samples with both values equal to or greater than 0.080 g/100 mL, the difference between the two analysis results from duplicate samples must agree within 5% of the average. This variation will be calculated using the following equation:

$$[(\text{high value} - \text{low value}) / \text{average value}] \times 100$$

2. For samples with at least one value less than 0.080g/100 mL, the analysis results must agree within 0.004g/100 mL of each other.

C. Conditions for reanalysis

If a sample is re-prepared and/or re-analyzed, it must be documented in the case record along with the reason.

1. If the analyst determines during the analysis session that the failure of a sample may be related to an isolated incident, the sample may be re-analyzed at the end of the analysis session. If resampled, it must be prepared with the same internal standard lot used for calibration and bracketed by ethanol controls, the final control must be an 0.080g/100 mL.
2. If the results do not meet the above specifications, the sample preparation and analysis must be repeated in a new analysis session. If after re-analysis the results do not meet the above specification, the case must be discussed with the quality manager.
3. If the results from the re-analysis meet the above specifications, the new set of data should be used for reporting. Results from each analysis session will be compared with each other to ensure the new results are reasonable. Both sets of chromatograms will be retained in the case record.

7.2 Reporting Ethanol Concentration

A. The average of the two results obtained will be truncated to three digits and reported. The two obtained results will be entered into LIMS. If ethanol is not detected on both chromatographic columns, enter zero (0) in the LIMS.

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

   "The reported value of concentration of alcohol in serum/plasma has not been converted to approximate the concentration of alcohol in whole blood."
3. For urine, LIMS will multiply the average of the two obtained results by 0.67, truncate to three digits, and report in grams of alcohol per 67 milliliters of urine.
4. For vitreous fluid, the results shall be reported in grams of alcohol per 100 milliliters and indicate the type of sample. A note shall be included on the report:

   "The concentration of alcohol in vitreous fluid is not equivalent to whole blood and should be interpreted with caution."
5. For other biological specimens, the results will indicate alcohol was detected. A note shall be included on the report:

   "Quantitation was not reported due to **[reason provided by the analyst]**."

B. If less than 0.010 g/100 mL (or g/67 mL for urine) is detected, but greater than or equal to 0.005 g/100 mL (or g/67 mL for urine), report "Less than 0.010 grams of alcohol per 100 milliliters of blood [67 milliliters of urine]". This is applied after any calculated conversions (e.g., urine conversion).

C. If greater than 0.500 g/100 mL (truncated to three decimals) is detected, report "Greater than 0.500 grams of alcohol per 100 milliliters of blood".

D. If the final ethanol result is less than 0.005 g/100 mL (or g/67 mL for urine) the report will indicate "No Alcohol Detected". This is applied after any calculated conversions (e.g. urine conversion).

E. If a dilution is performed, the requisite dilution correction is performed after the evaluation of the raw data. If the unadjusted average concentration

1. Falls within 0.010 and 0.500g/100 mL, report "[average concentration x 4] grams of alcohol per 100 milliliters of blood".
2. Is less than 0.010 g/100 mL, but greater than or equal to 0.005 g/100 mL, report "less than [low calibrator concentration x 4] grams of alcohol per 100 milliliters of blood".
3. Is greater than 0.500 g/100 mL (truncated to three decimals), report "Greater than 0.500 grams of alcohol per 100 milliliters of blood".
4. Is less than 0.005 g/100 mL, the report will indicate "No Alcohol Detected".

F. If there is insufficient sample (less than the normal sampling volume) to determine the ethanol concentration, the report should indicate "Insufficient sample for analysis" or an equivalent statement.

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

7.3 Evaluation and Reporting of Other Volatile Substances

A. The following volatile substances detected in a sample on both columns in any amount will be reported:

1. formaldehyde
2. toluene

B. The following volatile substances detected in a sample on both columns above either the recommended cut-off value of 0.005 g/100 mL or a lab's established cut-off value will be reported. If a lab is not using the recommended cut-off value, the lab's cut-off value will be documented in local policy.

1. acetone
2. isopropanol
3. methanol

The relative sample volatile response may be estimated by comparison of the responses of the sample volatile to the respective volatile responses in a volatile standard mixture or individual volatile standard, which is a known concentration.

C. Volatile substances detected on only one column and being reported 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.

D. A volatile mixture standard containing that volatile or an individual volatile standard must be tested at the time of analysis for comparison to the detected volatile in the sample. The

retention time of the detected peaks in the sample must be within 2% of the respective volatile standard.

E. The sample will be analyzed for toluene if:

1. requested by the submitter (e.g., inhalants or paint thinner), or
2. suspected by the analyst.

Note: retention time of toluene must be verified prior to completion of analysis in order to be reported.

F. If a volatile(s) is detected, it will be noted on the report as "[**Name of volatile**] detected."

G. If a volatile is observed on both columns and cannot be identified, then it is the analyst's discretion to note on the report, "A volatile was detected but not positively identified."

H. If a volatile is suspected by the customer but not detected by the laboratory, it will be noted on the report as "No other volatile detected."

7.4 Additional Reporting Notes

Any other circumstance regarding results that may warrant a result note will need to have the note approved by the Toxicology (Alcohol/Volatiles) advisory board before it is reported.

8 Records

Batch Record

LAB-201 – Laboratory Submission Form

LAB-203 – Toxicology Request Submission Form

LAB-BA-01 – Toxicology (Alcohol/Volatiles) Analysis Worksheet or LIMS equivalent

9 Literature References 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.

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

Forensic Toxicology Laboratory Guidelines, 2006 version; SOFT / AAFS.

BA-02-02 MEASUREMENT UNCERTAINTY ESTIMATE

1 Scope

To describe the uncertainty of measure determined by the Crime Lab Service for ethanol content in biological specimens.

2 Related Documents

BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis

CLS Manual – Laboratory Equipment

CLS Manual – Validations and Performance Verifications

3 Standards, Controls, and Calibration

NIST Traceable Ethanol Reference Standards

4 Practice

A. A system-wide measurement uncertainty percentage, which conveys approximately 99.7% confidence (coverage factor k=3), was determined from data across all laboratories at Texas DPS with a combined measurement uncertainty budget. The measurement uncertainty percentage is included in the case record and/or LIMS, such that it can be applied to the reported concentration of ethanol.

B. The reported concentration of ethanol is multiplied by the system-wide measurement uncertainty percentage to determine the estimate at that concentration. The calculated measurement uncertainty estimate is rounded up to three digits and is expressed as the reported concentration plus or minus the associated uncertainty in grams per 100 milliliters.

C. Current uncertainty estimate

   1. Uncertainty conversion factor: 4.3%, coverage factor k=3 and reported as 99.7% confidence interval

   2. Calculation of uncertainty estimate converted from the test value using the conversion factor to g/100 mL units

D. For example a reported concentration of 0.080 grams per 100 milliliters and an uncertainty percentage of 4.3% would be calculated and expressed as follows:

   (0.080) x 4.3% = 0.0034

   0.0034 rounded up to 3 digits = 0.004

   0.080 +/- 0.004 g/100 mL (reported concentration +/- calculated uncertainty estimate)

BA-02-03 INSTRUCTIONS FOR USING THE HAMILTON MICROLAB®

1 Scope

This document provides instructions for using the Hamilton Microlab® for sample preparation in Toxicology (Alcohol/Volatiles) analysis. It is assumed that a method is already programmed in the Microlab®.

Instructions in the document may need to be adjusted depending on the model used and each laboratory's specific programmed method.

2 Procedure

2.1 Program Instructions

Programming of the Microlab® is beyond the scope of this document. For instructions on programming the Microlab®, see The Microlab® User's Manual. (See co*nfigurations in this document.)*

2.2 Priming the Microlab®:

1. Turn on the Microlab® diluter/dispenser.
2. Fill diluent bottle with reagent (0.016 g/100 mL NaCl/n-Propanol internal standard solution).
3. Ensure the end of the tubing is in diluent bottle.
4. Aim the dispenser probe toward the waste container, then press the lower part of the **"STEP/PRIME"** switch on the drive unit. A programmed priming method may be used.
5. Allow syringes to flush until the fluid path is free of air bubbles.
6. If you do not see air bubbles, the system is ready for use – return **"STEP/PRIME"** switch to the middle position. Proceed to use Microlab® diluter/dispenser.
7. If the system is not airtight and the fluid path has air bubbles, adjust tubing fittings and syringes as necessary ensuring parts are finger-tight.
8. If air bubbles are still present in fluid path, remove tubing from diluent bottle and repeat steps 3-5 (prime with no liquid to dry the tubing).
9. Replace tubing into the diluent bottle and repeat priming with liquid.

2.3 Use of Microlab® for Analysis

1. From the main menu, select "**RUN AN EXISTING METHOD**".
2. Select the programmed **1-7** d**ilution** method. This method must be pre-programmed during validation.
3. A message about the syringe size used will appear. Visually confirm the display screen reads left syringe size is 2500 µL and right syringe size is 250 µL. Press the arrow pointed at "**CONFIRM**".

4. The screen should appear similar with the following recommended steps, volumes, and speeds:

```
      Dilute method: 1-7 DILUTI
Auto Dly Vol---LEFT-Spd Vol--RIGHT-Spd
1: Y       Fil   1400.0    4
2: Y       Dis                Asp
3: N                          Asp   200.0    2
4: N       Dis   1400.0    7  Dis   200.0    2
5: Y       Fil   1000.0    3
6: Y       Dis
7: N       Dis   1000.0    2
    RUN/Probe to start. ESCAPE to exit
```

		ESCAPE		

5. Pipette samples (calibrators, standards or case samples) by placing the dispenser probe tip into specimen and press the black button on the dispenser probe to aspirate sample. Clean the outside of the probe tip with a disposable wipe.
6. Place the dispenser probe inside and touching the side of the appropriately labeled headspace vial, then press the black button a second time to dispense specimen and internal standard into the vial.
7. Aim the dispenser probe toward the waste container and press the black button again to dispense the wash. Wash the outside of the probe with purified water and dry the probe tip with a disposable wipe.
8. Repeat steps 5-7 for all samples. When pipetting of all samples is complete, press the arrow pointed at "**ESCAPE**" to return to the main menu.

2.4 Microlab® shut-down procedure:

1. Replace internal standard diluent bottle with bottle of purified water.
2. Aim the dispenser probe toward the waste container, then press the lower part of the "**STEP/PRIME**" switch on the drive unit. A programmed priming method may be used.
3. Flush the syringe approximately 10 times to ensure complete rinsing.
4. When priming is complete, press the upper part of the "**STEP/PRIME**" switch on the drive unit until the syringe is filled with purified water.

   <u>Note</u>: It is important to leave the diluent syringe and tubing filled with purified water to prevent damage from salt.
5. Turn off the Microlab® diluter/dispenser.

3 Literature References and Supporting Documentation

The Microlab® 500 Series User's Manual, Hamilton Company, Reno, Nevada, 1999. (or user's manual for current model)

BA-02-04 VALIDATION OF SIGNIFICANT EQUIPMENT

1 Scope

Validations will be conducted to assess the equipment's reliability to produce quality results prior to use on casework incorporating applicable methodology used for analysis. This document outlines minimum requirements for conducting validations.

Any study performed for an equipment validation that includes testing a matrix sample must evaluate both a blood and a urine sample.

2 Related Documents

BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis

CLS Manual – Validations and Performance Verifications

3 Practices

3.1 Validation of a New Model of Gas Chromatograph

A. Single Volatiles –

1. Analyze a volatile standard mix to ensure separation between the peaks.
2. Analyze individually acetaldehyde, ethanol, formaldehyde, methanol, isopropanol, acetone, toluene, and n-propanol to determine the retention times.

B. Calibration Model – Prepare a calibration curve using calibrators from the established method.

Evaluation Criteria: Calibration must meet all method criteria.

C. Limit of Quantitation –

1. Analyze an external ethanol standard of 0.010 g/100 mL prepared with internal standard.
2. At least three analysis sessions with three samples will be run.

Evaluation Criteria: The concentration of each sample should be +/- 20% of the target value.

D. Limit of Detection –

1. The LOD is the administratively defined decision point, which is 0.005 g/100 mL.
2. At least three analysis sessions with three samples will be run.

Evaluation Criteria: Ensure that the signal-to-noise ratio is greater than 3:1 and meets all detection criteria.

E. Carryover Study –

1. Prepare or purchase a 1.0 g/100 mL ethanol solution. If preparing, weigh out 1.0 g of 100% or 95% ethanol and place it in a 100 mL volumetric flask that is filled with purified water using proper laboratory techniques.
2. Prepare a 0.001 g/100 mL ethanol solution by weighing out 0.001 g of 100% or 95% ethanol and placing it in a 100 mL volumetric flask that is filled with purified water using proper laboratory techniques. Alternatively, a purchased standard may be diluted.

3. Sample and analyze (200 µL solution and 1400 µL purified water) the solutions in the following order:
   a) *Blank*
   b) *0.001 g/100 mL*
   c) *Blank*
   d) *1.0 g/100 mL*
   e) *Blank*

Evaluation Criteria: The ethanol peak area in the blank sample succeeding the 1.0 g/100 mL solution must not exceed the peak area for ethanol in the 0.001 g/100 mL solution.

F. Repeatability – Sample and analyze a 0.08 g/100 mL standard at least ten times.

Evaluation Criteria: Calculate the mean, standard deviation, and coefficient of variation. The mean must be within 5% of the expected value. The coefficient of variation must be under 5%.

3.2 Validation of a New Model of Automatic Diluter/Dispenser

Note: Labs only using automatic diluter/dispenser to dispense internal standard solution need only follow step A.2.

A. Gravimetric Study – Sample and dispense the following volumes into a clean container and weigh on a balance:

1. 0.2 mL using the small syringe at least 10 times
2. 1.4 mL using the large syringe at least 10 times
3. 1.6 mL using lab's sampling method at least 10 times

Evaluation Criteria: Must meet manufacturer's specifications for accuracy. The coefficient of variation must be within 1%.

B. Carryover Study – Sample and analyze high ethanol concentration solutions followed by an aqueous blank:

1. Aqueous: Sample a purchased or prepared aqueous solution of a concentration at or above the highest ethanol concentration standard run in casework
2. Specimen Matrix: Sample a prepared matrix solution at an approximate ethanol concentration of 1.0 g/100 mL

Evaluation Criteria: Ethanol peak area in aqueous blanks must not exceed gas chromatograph's LOD

4 Literature References and Supporting Documentation

SWGTOX Method Validation Recommendations, May 2013.

ISO/IEC 17025:2017

03 STANDARDS AND SOLUTIONS

BA-03-01 PREPARATION OF NaCl/n-PROPANOL INTERNAL STANDARD SOLUTION

1 Scope

The combination of both sodium chloride (NaCl) in aqueous form and npPropanol in the same solution enhances the efficiency of preparation of samples. The addition of NaCl to headspace vials increases the sensitivity of the method. The n-propanol is added as an internal standard which facilitates accurate determination of the quantity of ethanol.

2 Specification

NaCl/n-Propanol Solution, NaCl/n-Propanol Internal Standard Solution

3 Safety

Basic laboratory safety precautions. Familiarity with chemical hazards as listed in Safety Data Sheets (SDS).

4 Equipment and Materials

- Volumetric flask
- Volumetric TD (to deliver) pipettes
- Positive displacement pipettes
- Balance
- NaCl
- n-Propanol
- Purified water
- Sodium Azide

5 Instructions

A. For NaCl/n-Propanol internal standard solution (0.016 g/100 mL)

1. In a volumetric flask, dissolve the following components in the respective ratio: Add 500 g NaCl, 0.32 g (or 400 µL) n-propanol, and bring to 2000 mL with purified water.

   Optionally, the quantity of NaCl added may be reduced up to 50% if the lab performs an implementation validation.

2. Addition of 13 g sodium azide (optional).

B. Minimum labeling includes identity and either date of preparation or lot number of the solution on the container. Preparer's identity and components used in preparation must be recorded and maintained.

6 Testing, Storage, Expiration, and Disposal

A. Reliability testing shall occur prior to use.

1. Minimum acceptance criteria:

   a) Retention time must be within 2% of the previously verified retention time for n-propanol, and

Printed copy is uncontrolled. Refer to electronic copy for current version.

b) No detectable volatiles other than n-propanol

2. If the reagent does not meet criteria

a) Attempt to determine cause of discrepancy, perform necessary corrective action(s), and retest reagent

b) If the discrepancy cannot be resolved the reagent will not be used and a new internal standard solution must be prepared

B. Solution may be stored at room temperature. Shelf life for solution is one year.

BA-03-02 PREPARATION OF VOLATILE MIXTURE STANDARD

1 Scope

The volatile mixture standard is used in the analysis procedure to validate the separation of various volatile substances and potential contaminants from ethanol.

2 Specification

Volatile mixture standard solution

3 Safety

Basic laboratory safety precautions. Familiarity with chemical hazards as listed in Safety Data Sheets (SDS).

4 Equipment and Materials

- Volumetric flasks
- Volumetric pipettes or pipettors
- Disposable pipettes
- Acetone
- 95% Ethanol
- 200 proof Ethanol
- Isopropanol
- Formaldehyde solution, or Formalin, (contains 10-15 % Methanol)
- Acetaldehyde
- Toluene
- Purified water

5 Instructions

A. In a volumetric flask, dilute the following components in the approximate ratio with purified water.

B. For a 100-mL volumetric flask (volume ratio):

1. 64 µL acetone,
2. 127 µL isopropanol,
3. 1500 to 5000 µL formaldehyde solution,
4. 50 µL acetaldehyde,
5. 46 µL toluene (optional), and
6. 133 µL 95% ethanol or 125 µL 200 proof ethanol

C. For the volume ratios listed above, the approximate concentration in grams per 100 mL would be as follows:

1. Acetone= 0.05 g/100 mL
2. Isopropanol= 0.10 g/100 mL

3. Methanol= between 0.12 and 0.60 g/100 mL, depending on:
   a) *Percent methanol in formaldehyde solution*
   b) *Volume formaldehyde solution added*
4. Ethanol= 0.10 g/100 mL

   The concentrations are determined using the following calculation:

   Volume added (mL) x density of the volatile

D. Minimum labeling includes identity and either date of preparation or lot number of the solution on the container. Preparer's identity and components used in preparation must be recorded and maintained.

6 Testing, Storage, Expiration, and Disposal

A. Reliability testing shall occur prior to use.
   1. Minimum acceptance criteria:
      a) *Individual components are separated and identified*
      b) *Retention times must be within 2% of the previously verified retention time for each individual component*
   2. If the reagent does not meet criteria
      a) *Attempt to determine cause of discrepancy, perform necessary corrective action(s), and retest reagent*
      b) *If the discrepancy cannot be resolved the reagent will not be used and a new volatile mixture standard must be prepared*

B. Store at room temperature or refrigerate. Shelf life is determined by loss of components or up to 1 year.

7 Literature References and Supporting Documentation

Siek, T. J., 1971. Duplicate analysis of blood ethanol by injection onto two parallel gas chromatographic columns in rapid succession. *Journal of Forensic Sciences*, 334-339.

Solon, J., J. Watkins, and L. Mikkelsen. 1971. Automated analysis of alcohols in blood. *Journal of Forensic Sciences*, 445-452.

04 FORMS

DIRECTORY OF FORMS

	Document Name	FRN
1	Toxicology (Alcohol/Volatiles) Analysis Worksheet	LAB-BA-01