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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



01 TOXICOLOGY (ALCOHOL/VOLATILES) TRAINING UNIT

BA-TM-01-01 TRAINING UNIT OVERVIEW

1 Introduction

Approval for Toxicology (Alcohol/Volatiles) analysis includes the analysis of blood, urine, vitreous, and other biological specimens.

Individuals must meet specific qualifications as outlined in the Toxicology (Alcohol/Volatiles) Training Manual before being qualified to perform casework. The qualifications consist of educational requirements and forensic experience requirements.

2 Purpose

For personnel meeting the minimum DPS educational employment requirements for the position, the Toxicology (Alcohol/Volatiles) Training Manual is designed to provide the trainee with sufficient background, laboratory skills, education, competency, and supervised hands-on experience to adequately perform independent casework with minimal mentoring. The training program is identified as containing a set of required modules. The suggested duration of each training module is an estimation and may vary based on the trainee's previous experience, trainer's schedule, and other factors.

3 Program Format

- A. The Texas DPS training time is approximately one to six months. Trainees having prior experience in Toxicology (Alcohol/Volatiles or Drugs) analysis procedures of biological specimens may be evaluated to facilitate the individual's training time and program. Any modifications to the Toxicology (Alcohol/Volatiles) Training Manual must be documented in a memo and approved by Quality Assurance and the Toxicology (Alcohol/Volatiles) Advisory Board chair.
- B. General Laboratory Training – The trainee will be introduced to general laboratory practices, forensic science, quality assurance, evidence management, law, pretrial preparation, court testimony, measurement uncertainty, and general laboratory safety. As part of the Toxicology (Alcohol/Volatiles) training program, the trainee will be introduced to safety, basic equipment use, sample preparation, report writing and case review.
- C. Toxicology (Alcohol/Volatiles) Analysis Training – The trainee will be introduced to chemical properties, physiology and pharmacology, fundamental operations, headspace gas chromatography, and analytical procedures for Toxicology (Alcohol/Volatiles) analysis, including result evaluation.

4 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-02 – Safety.

5 Responsibilities

5.1 Assignment of Trainer

- A. Prior to beginning the training process, the trainer and the supervisor should evaluate the trainee's previous training and experience for possible modifications to the training plan.
- B. Meetings between the trainee, the trainer, and/or supervisor should be held periodically in order to evaluate the trainee's progress, plan future study/practical assignments and



discuss any deficiencies, which require additional training. The trainer will document the trainee's progress.

5.2 Trainee Responsibilities

- A. The training program covers information that requires the trainee to complete reading assignments on a self-study basis.
- B. The trainee shall take notes summarizing assigned readings and videos, and review as necessary for subsequent modules and/or quizzes/exams.
- C. The trainee will be required to keep detailed records of his/her training in a training notebook.
- D. The trainee will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01) of required and optional elements of training.
- E. The trainee is responsible for informing his/her trainer or supervisor when problems arise at any time during the training period.

5.3 Trainer Responsibilities

- A. The trainer is responsible for providing a training plan or outline to the trainee.
- B. Completion of tasks by which the trainee is assessed, modules and/or practical exercises will be evaluated, dated, and signed by the trainer.
- C. The trainer will review and approve the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- D. The training notebooks, other training records documenting completion of training requirements, and trainee's credentials are reviewed and approved by the trainer.

5.4 Required Readings

Prior to the start of training, the trainee will review the Employee Training Program chapter of the CLS Manual, specifically sections covering

- A. On-the-Job Training
- B. Acceptable Performance

6 Training Resources

- A. All required readings or videos are available at either the training laboratory or in the Laboratory Resources/E-Train Collection on SharePoint at the QA site, under the "BA Training" view.
- B. For literature references, use of the most current edition is recommended.

7 Required External Training Courses

Trainee shall complete the following course within one year of obtaining independent work authorization:

Indiana University – The Robert F. Borkenstein Course on Alcohol and Highway Safety: Testing, Research, and Litigation



8 Review and Authorization

8.1 Unit Assessment

- A. Unit training assessment will be undertaken as separate modules of training.
- Exercises and review quizzes are an evaluation of the trainee's understanding of each module and are not subject to pass/fail criteria. Results and/or answers will be reviewed by the trainer, feedback given to the trainee and additional exercises or quizzes may be given at the discretion of the trainer.
- B. Conclusion of the unit is accomplished when:
1. The trainee independently examines at least fifteen (15) unknown biological samples for competency.
 2. The trainee successfully completes the approved comprehensive standardized written examination for Toxicology (Alcohol/Volatiles). The score on the written exam must be 75% or better to pass.
 3. The trainee will complete a mock trial before the commencement of supervised casework.
- C. The training notebook, other training records documenting completion of training requirements, and trainee's credentials are reviewed by Quality Assurance or the Quality Manager.

8.2 Work Authorization

- A. Upon completion of the training unit, the trainee may begin performing supervised casework with the approval of the Laboratory Director, using the Work Authorization form (LAB-309).
- B. Supervised casework requirements will conclude with independent examiner approval when the following are met:
1. The trainee will complete at least two batches of casework under supervision.
 2. The trainer(s) recommend that the trainee be approved for independent casework to the Quality Manager. The Quality Manager approves the trainee to conduct independent casework by signing the Work Authorization form (LAB-309).



BA-TM-01-02 SAFETY

Duration 2 days

Purpose To orient and acquaint the trainee with Toxicology (Alcohol/Volatiles) specific safety practices to be followed in the laboratory

Prerequisite GLT-TM-FUN-01 to 06

1 Objectives

1.1 Theoretical

Safe handling of samples and the waste generated during analysis is critical to protect the health of all persons who may come into contact with samples or waste either within or outside of the laboratory. Safety training promotes healthy employees and minimizes liability to the agency.

1.2 Practical

Following the completion of this module, the trainee will:

- A. Be aware of specific hazards associated with performing Toxicology (Alcohol/Volatiles) analysis.
- B. Be able to locate and use SDS for chemicals used in analysis.
- C. Understand and practice **universal precautions** for blood and other biological specimens, including use of required personal protective equipment (PPE).
- D. Know local work practice controls such as how to properly handle and dispose of biological contaminated supplies and properly disinfect the work areas.
- E. Be familiar with local engineering controls such as hoods, chemical storage cabinets, chemical labeling and disposal, proper handling of gas cylinders, use of gas generators, and waste disposal systems.

2 Training Outline

2.1 Lesson Plan

- A. Bloodborne pathogens review
- B. Protective measures review
 1. Universal precautions
 2. Required PPE
 3. Hoods and snorkels
 4. Chemical storage cabinets location and use
 5. Pressurized gas cylinders
 6. Pressurized air outlets
 7. Gas generators
 8. Glass handling
 9. Electrical
 10. Flammable gas



- C. Preventative practices for procedural risks
 - 1. Avoid contamination of documents and personal items
 - 2. Cutting seals (box cutter safety)
 - 3. Homogenizing sample using glass tissue grinder and proper clean-up
 - 4. Avoiding exposure resulting from pressure buildup in specimen containers
 - 5. SDS of chemicals used in Toxicology (Alcohol/Volatiles) procedures
- D. Waste practices
 - 1. Disposable pipette tips, headspace vials, gloves
 - 2. Laundry of lab coats
 - 3. Autoclave
 - 4. Local waste disposal policies
- E. Emergency
 - 1. Fire safety
 - 2. Spills
- F. Shipping guidelines for biological specimens, including proper packaging

2.2 Required Readings

- A. Safety Data Sheets
Review for each solvent, drug and chemical used.
- B. If more than 6 months since trainee has completed GLT-TM-FUN-02 (General Safety), trainee should review the Biological Pathogen Exposure Control Plan chapter of the Safety Manual. Alternatively, the trainee can view one or more videos covering the topic of bloodborne pathogens.
- C. Shipping guidelines for biological specimens
 - 1. CLS Manual – Toxicology (Alcohol/Volatiles and/or Drugs) Analysis
 - 2. United States Postal Service –
https://pe.usps.com/text/pub52/pub52apxc_022.htm#ep1000377
 - 3. FedEx –
http://www.fedex.com/us/services/pdf/How_To_Pack.pdf pages 31-34

2.3 Suggested Videos

Note: these videos are meant to be supplementary to the General Training safety module, and may be used at trainer's discretion.

- A. Pro Bloodborne: Bloodborne Pathogens training – <http://www.probloodborne.com/en/training>
 - 1. Types of Bloodborne Diseases
 - a) *HIV and AIDS*
 - b) *Hepatitis B Virus*
 - c) *Hepatitis C Virus*



2. Engineering and Work Practice Controls
 - a) *How to Reduce Your Risk*
 - b) *Work Practice and Engineering Controls*
 - c) *Regulated Waste*
 - d) *Body Fluid Cleanup*
 - e) *Glove Removal*
 - f) *Handwashing*

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions:

- A. Wear gloves, a lab coat, and protective eyewear when working with biological specimens or during reagent preparation and testing. Proper gowning technique (gloves overlapping the sleeves) must be followed.
- B. Clothing may protect unbroken skin; broken skin should be covered by a protective bandage.
- C. The biological safety cabinet (laminar flow hood, biohood) should be used when handling potentially hazardous biological samples. Contents of the hood must not interfere with circulation.
- D. Conventional fume hoods should be used when handling volatile chemicals during reagent preparation.
- E. Use universal precautions during evidence handling. Care should be exercised during the use of cutting tools while handling evidence.
- F. Hydrogen is a highly flammable gas used under pressure for gas chromatography. Leaks and proper cylinder handling are primary concerns.
- G. Heated zones of the gas chromatograph require caution to avoid burns.

3.2 Observed Performance

- A. The trainer will lead trainee on tour of the lab, pointing out location of analysis materials and related safety equipment. Proper use, storage, and disposal will be discussed.

Note: *the following may be completed concurrent with later modules, at trainer's discretion*

- B. Trainee will observe when a pressurized gas cylinder is changed out.
- C. Trainer will discuss and demonstrate safety issues associated with the use of hydrogen gas when using a pressurized gas cylinder and/or hydrogen generator, as applicable.

3.3 Independent Exercises

- A. The trainee will complete an exercise demonstrating knowledge of use and location for analysis related safety equipment, devices, SDS, and local safety practices.
- B. The trainee will demonstrate to trainer the PPE to be worn during analysis and how to properly remove PPE.



4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-03 INTRODUCTION TO TOXICOLOGY (ALCOHOL/VOLATILES) ANALYSIS

Duration 1 week

Purpose To enable trainee to communicate using appropriate terminology and to educate trainee on the overall procedures of Toxicology (Alcohol/Volatiles) analysis.

Prerequisite BA-TM-01-02, GLT-TM-LAW-01, GLT-TM-LAW-02, GLT-TM-LAW-02A

1 Objectives

1.1 Theoretical

Understanding the techniques used in Toxicology (Alcohol/Volatiles) analysis provides a foundation for the training program. The ability to understand and use proper terminology is necessary for successful communication of Toxicology (Alcohol/Volatiles) analysis in court.

This module will provide an overall view of how Toxicology (Alcohol/Volatiles) analysis is accomplished for DWI and other alcohol related offenses by introducing basic concepts related to the testing of alcohol and other volatiles. Subsequent modules will explore these concepts in more detail.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Define and understand basic forensic chemistry terms.
- B. Understand the function and responsibilities of the Toxicology (Alcohol/Volatiles) section.
- C. Name our accrediting bodies, what they do and how DPS is accredited.
- D. List the various specimen types.
- E. Have a basic working knowledge of the various types of sampling and testing techniques.
- F. Understand the calibration process.
- G. Understand how Henry's law governs our forensic technique.
- H. Understand the basics of Justice Trax[®], our reporting system.
- I. Summarize the Toxicology (Alcohol/Volatiles) workflow from submission of evidence to generation of reports.
- J. Understand the Texas laws related to intoxication and alcoholic offenses.
- K. Understand case laws relevant to analysis and testimony.

2 Training Outline

2.1 Lesson Plan

Topics will be addressed through readings and/or discussion with trainer

- A. Glossary terms and approved abbreviations, including
 1. Ethanol chemistry
 2. Sampling, specimen types and matrices
 3. Instrumentation



4. Laws and law related terms
 5. General Manual and Crime Laboratory Service Manual alcohol related terms
 6. Evidence receiving
 7. General scientific terms
- B. DPS Crime Laboratory System mission, goals, responsibilities, and objectives
- C. Accreditation process
1. Locate and read the accrediting certificate and memo and know your laboratory's scope of service.
 2. Briefly go over goals and/or missions of SOFT, ANAB, FSC, OSAC and other pertinent organizations.
 3. Read the National Academy of Sciences (NAS) summary report.
- D. Specimen types
1. Blood
 2. Serum/Plasma
 3. Urine
 4. Breath
 5. Other tissues
- E. Ethanol analysis
1. Types of blood/fluid analysis
 - a) *Chemical reactions*
 - b) *Biochemical*
 - c) *Instrumental*
 2. Other types of analysis
 - a) *Breath Analysis (quantitative)*
 - b) *Breath Analysis (qualitative)*
- F. Instrument calibration
1. Single point
 2. Multi-point
- G. Review the principles of Henry's law and its relevance with heated headspace analysis
- H. Introduction to the LIMS system, including demonstration of Justice Trax[®]
1. Evidence tracking
 2. Input of data
 3. Final reports

**I. Texas Laws**

1. Penal Code (intoxication and alcoholic offenses), *Chapter 49, Sections 49.01 through 49.09*
2. Transportation Code
 - a) *Chapter 524: Administrative Suspension of Driver's License, Sec. 524.001 to 524.023*
 - b) *Chapter 724: Implied Consent, Sections 724.001 to 724.019*
3. Alcoholic Beverage Code, *Chapter 106: Provisions relating to age, Section 106.01 to 106.05*

J. Case law, including but not limited to

1. *Daubert v. Merrell Dow Pharmaceuticals* (1993)
2. *Raul Mata v. Texas* (2004)
3. *Paredes v. Texas* (2015)
4. *Talamantes v. State of Texas* (2015)

2.2 Required Readings

Readings that have been completed as part of General Laboratory Training may be omitted or reviewed at trainer's discretion.

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedures,
 1. BA-01-05 – Approved Abbreviations
 2. BA-01-06 – Glossary of Terms
- B. Crime Laboratory Service Manual (CLS),
 1. Scope (Standard 1 – 17025:2017; ANAB AR 3125)
 2. Terms and Definitions (Standard 3 – 17025; ANAB AR 3125)
 3. Introduction
 4. General Laboratory Information
 5. Laboratory Service Requests
 6. Required Forms and Evidence Collection Kits
 7. Laboratory System Roles and Responsibilities
 8. Laboratory Code of Ethics
- C. Levine, Barry, ed. *Principles of Forensic Toxicology*. 4th ed. Revised. Washington, D.C.: American Association of Clinical Chemistry. 2013. Chapter 13 – Alcohol
- D. Accreditation and organizations readings:
 1. Forensic Science Commission: <http://www.fsc.texas.gov/about>
 2. ANSI National Accreditation Board (ANAB):
<https://www.anab.org/about-anab>
 3. Society of Forensic Toxicologists (SOFT) brochure



4. The Organization of Scientific Area Committees for Forensic Science (OSAC) information:
<https://www.nist.gov/topics/forensic-science/osac-organizational-structure>
5. National Academy of Sciences (NAS) Report, "Strengthening Forensic Science in the United States: A Path Forward." 2009. Summary section.
- E. Garriott, James C. ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015.
 1. Chapter 5 – Specimen Considerations for Alcohol Testing, *sections 5.1 - 5.12, pp 195-206*
 2. Chapter 7 – Methods for Breath Analysis, *sections 7.1 - 7.4, pp 229-234.*
 3. Chapter 8 – Methods for Fluid Analysis, *sections 8.1 - 8.3, pp 253-257*
- F. Levine, Barry, ed. Principles of Forensic Toxicology. 4th ed. Revised. Washington, D.C.: American Association of Clinical Chemistry. 2013. Chapter 9 – Chromatography, *Quantitation section, pp. 144-148*
- G. LIMS Manual,
 1. LIMS-ADM-01 – Laboratory Information Management System
 2. LIMS-ADM-05 – Hardware and Software
 3. LIMS-ADM-06 – LIMS Support Assistance
- H. Texas laws outlined in section 2.1.I.
Note: Access to all current codes and statues for this module are located:
<http://www.statutes.legis.state.tx.us/>
- I. "Selection of DWI Court Decisions through August 2011"
- J. Case laws outlined in section 2.1.J.

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).

3.2 Observed Performance

Trainee will observe a qualified analyst perform a batch run from start to finish including: assigning cases, sampling of evidence, how to prepare the instrument for a batch run/sequence, and report writing in the LIMS computer system.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).



- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-04 CHEMICAL PROPERTIES OF ETHANOL

Duration 2 to 5 days

Purpose To familiarize the trainee with the chemistry, history, and production of ethanol.

Prerequisite BA-TM-01-03

1 Objectives

1.1 Theoretical

For thousands of years alcohol has been an active component in societies. Only in recent times have we studied how alcoholic beverages are processed to such a degree that we can produce any percent alcohol we like and flavor it as well. Ethanol is produced by fermentation: yeast consume sugar or starch from fruits and grains and excrete ethanol. Alcoholic beverage industry standards now exist to define fermentation products according to alcohol content, and the trainee must be familiar with these terms and processes.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Draw the structure of ethanol.
- B. Explain the chemical properties of ethanol.
- C. List and define the different terms used for ethanol.
- D. Describe the fermentation process of sugar or starch into ethanol.
- E. Summarize the history of ethanol production for human consumption.
- F. Explain the difference between proof and percent.
- G. Briefly describe how beer, wine, and liquor are produced.
- H. Know the approximate percent content of beer, wine, and liquor.
- I. Describe a standard drink in layman's terms.
- J. Classify congeners and their role in alcoholic beverages.

2 Training Outline

2.1 Lesson Plan

- A. Ethanol
 1. Chemical structure of ethanol
 2. Chemical properties of ethanol
- B. History of ethanol production
- C. Production of ethanol
 1. Fermentation process
 2. Alcoholic beverages
 - a) *Proof*
 - b) *Percent content*
 - c) *Congeners*



2.2 Required Readings

- A. Texas Breath Alcohol Testing Program Operator Manual, current edition. Ethanol Chapter
- B. Garriott, James C., ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 1 – Chemistry of Alcoholic Beverages

3 Practice

3.1 Safety

None

3.2 Independent Exercises

The trainee will create summary notes over information in the required readings.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LA-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-05 PHYSIOLOGY AND PHARMACOLOGY OF ETHANOL

Duration 2 to 4 weeks

Purpose To familiarize the student with the absorption, distribution, and elimination of ethanol within the body, and the effects of ethanol on the human body.

Prerequisite BA-TM-01-03, BA-TM-01-04

1 Objectives

1.1 Theoretical

Rule 702 of the Federal Rules of evidence states “If scientific... knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise.”

To be able to render an opinion, the forensic scientist must determine the concentration of ethanol in the specimen, and make any conversions appropriate to the type of specimen. Then the scientist can assist a “trier of fact” by explaining the stage of alcoholic influence of an average person at a specified blood alcohol concentration. The forensic scientist must also explain the impact or significance of other volatiles and compare blood alcohol results to those from other body specimens.

1.2 Practical

The student will become familiar with the effects of ethanol on the human body, to include observed effects, alcohol absorption, distribution, and elimination. Blood alcohol and its relationship to alcohol in other specimens are discussed, as are the presence and effects of other volatiles on the body.

Following the completion of this module, the trainee will be able to:

- A. Describe the pharmacological effects of ethanol on the different parts of the body.
- B. Be familiar with the pharmacological presence and effects of other volatiles on the body.
- C. Know the lethal dose of ethanol in the human body.
- D. Describe the stages of acute alcoholic influence/intoxication.
- E. Be familiar with effects of alcohol in combination with other drugs.
- F. Define tolerance and give examples of each type.
- G. Know the decomposition products of ethanol in the human body.
- H. Describe the passage of ethanol through the body starting with the mouth.
- I. Describe absorption, distribution and elimination.
- J. Be familiar with the different metabolic pathways for ethanol in the human body.
- K. Know the ratios of whole blood to other specimen types.
- L. Know the units used to report ethanol in blood, serum/plasma, urine, and breath.
- M. Know the units for reporting ethanol used by hospitals.
- N. Understand the basic instrumental theory of the breath testing program.



2 Training Outline

2.1 Lesson Plan

- A. Human Physiology Overview
- B. Ethanol: Ingestion to elimination from the body
 - 1. Absorption
 - 2. Distribution
 - 3. Elimination
 - 4. Biochemistry of metabolism
 - a) *Oxidation by alcohol dehydrogenase*
 - b) *Microsomal ethanol oxidizing system*
- C. Pharmacological effects of ethanol on the body
 - 1. Stages of influence and intoxication
 - 2. Tolerance
 - 3. Combination with other drugs
- D. Pharmacological presence and effects of other volatiles on the body
- E. Types of specimens, preservation, ratios and conversion factors, and reporting units
 - 1. Specimens
 - a) *Blood components (whole blood, serum, plasma)*
 - b) *Various specimens (blood, urine, vitreous, tissue)*
 - c) *Preservation of specimens*
 - i. *Microbial effects*
 - ii. *Preservatives*
 - iii. *Anticoagulants*
 - d) *Specimen age*
 - 2. Reporting units and ratios to whole blood
 - a) *DPS / Intoxication Statute*
 - b) *Serum/Plasma*

2.2 Required Readings

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedure, BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis,
 - 1. Section 7.2 – Reporting Ethanol Concentration (*subsection A only*)
 - 2. Section 7.3 – Evaluation and Reporting of Other Volatile Substances
- B. Review Texas Penal Code Chapter 49, section 49.01 – Definitions, *Subsection (1)*
- C. Saferstein, Richard, ed. *Forensic Science Handbook*. 2nd ed. New Jersey: Prentice-Hall, 2002. Chapter 12 – The Determination of Alcohol in Blood and Breath



- D. Garriott, James C. ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015.
1. Chapter 2 – Pharmacology and Toxicology of Alcohol
 2. Chapter 3 – Disposition and Fate of Ethanol in the Body,
 - a) Sec. 3.1 - 3.6.C, pp. 49-86
 - b) Sec. 3.7.N - 3.9, pp. 110-130
 3. Chapter 4 – Biomarkers of Acute and Chronic Alcohol Ingestion
 4. Chapter 5 – Specimen Considerations for Alcohol Testing, Sec. 5.13, pp. 206-209
 5. Chapter 6 – Physiological Basis and Practice of Breath Alcohol Determination
 6. Chapter 11 – Alcohol Effects and Driving Impairment
 7. Chapter 12 – Standardized Field Sobriety Testing
 8. Chapter 15 – The Manifestation of Alcohol Intoxication
- E. Baselt, Randall C, Disposition of Toxic Drugs & Chemicals in Man, 10th ed. Seal Beach, CA: Biomedical Publications, 2014. Sections on ethanol and other volatiles
- F. Moffat, Anthony C., Osselton, M. David, Widdop, Brian, Watts, Jo, eds. Clarke's Analysis of Drugs and Poisons, 4th ed. Gurnee, IL: Pharmaceutical Press, 2011. Chapter 10 – Postmortem Toxicology, pp. 176-179, pp. 184-185 (sections on Ethanol and Other Alcohols and Volatiles)
- G. Lieber, Charles S. "The Metabolism of Alcohol." Scientific American, 234.3 (1976): pp. 25 - 33
- H. Jones, A.W. and Andersson, L. "Influence of Age, Gender, and Blood-Alcohol Concentration on the Disappearance Rate of Alcohol from Blood in Drinking Drivers." Journal of Forensic Sciences 41.6 (1996): pp. 922-926
- I. Dubowski, Kurt M., "Absorption, Distribution, and Elimination of Alcohol: Highway Safety Aspects." Journal of Studies on Alcohol, Supplement No. 10 (1985): pp. 98-108
- J. Karch, Steven B. ed. Drug Abuse Handbook, 2nd ed. Boca Raton, FL: CRC Press, 2007. Section 14.1.1.3 – Rising Blood-Alcohol Concentration, pp. 1122-1124
- K. Harding P. Alcohol Toxicology for Prosecutors: Targeting Hardcore Impaired Drivers. Alexandria, VA: American Prosecutors Research Institute; 2003. Interpretation of alcohol results; pp. 1-23
- L. Wigmore, James G. Wigmore on Alcohol: Courtroom Alcohol Toxicology for the Medicolegal Professional. Toronto: Irwin Law, 2011. Chapter 1 – Absorption, Distribution, and Elimination of Alcohol
- M. Cowan, Mack. "Converting Serum to Blood Alcohol Concentration". PowerPoint presentation (2015)

2.3 Required Videos

- A. Physiology and Pharmacology of Alcohol: Forensic Science Aspects, Jones, Alan Wayne, Date of seminar: 2-19-94, (4 hours)
- B. *Drinking and Driving: Is 0.08 too late?* (30 min), Texas A&M ©2000 driving study



- C. *Alcohol and the Human Body* (10 min), Tarrant County College ©2001
- D. Texas A&M: The Effects of Alcohol on Driving, ©1993 (20 min)
- E. The Effects of Alcohol on Driving (the ALR video). Tarrant County Junior College Northeast, ©1987

2.4 Suggested Readings

- A. Wigmore, James G. Wigmore on Alcohol: Courtroom Alcohol Toxicology for the Medicolegal Professional. Toronto: Irwin Law, 2011. Chapter 5 – Effect of Alcohol on Driving, pp. 207-248
- B. Levine, Barry, ed. *Principles of Forensic Toxicology*. 4th ed. Revised. Washington, D.C.: American Association of Clinical Chemistry. 2013. Chapter 28 – Inhalants

3 Practice

3.1 Safety

While observing or participating in a Breath Alcohol program for controlled drinking, the trainee must follow all rules set forth by the program directors.

3.2 Independent Exercises

- A. The trainee will create summary notes over information in the required readings and videos.
- B. The trainee will observe a video of an intoxicated person (if available) and review the results of the Toxicology (Alcohol/Volatiles) analysis of that person to make a correlation between exhibiting symptoms and alcohol content.
- C. The trainee will attend a Breath Alcohol or Toxicology (Alcohol/Volatiles) study as soon as possible during the analyst's training if available, or no later than within the first year after training. If unavailable, observation of additional videos depicting alcohol impaired subjects may be done in lieu of attending live training. Alternately, direct observation of subjects being evaluated by officers and performing breath analysis for alcohol intoxication will suffice for this component.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-06 WIDMARK AND RETROGRADE EXTRAPOLATION

Duration 1 to 2 weeks

Purpose To familiarize the student with the calculations that they may utilize to estimate the number of alcoholic drinks an individual may have consumed or the blood alcohol concentration at a previous time.

Prerequisite BA-TM-01-03, BA-TM-01-04, BA-TM-01-05

1 Objectives

1.1 Theoretical

To be able to render an opinion, the forensic scientist must determine the concentration of ethanol in the specimen, and make any conversions appropriate to the type of specimen. Then the scientist can assist a “trier of fact” by explaining the stage of alcoholic influence of an average person at a specified blood alcohol concentration, and, given adequate information, to ascertain the estimated number of drinks required to reach a given concentration of ethanol.

1.2 Practical

Following the completion of this module, the trainee will:

- A. Know and utilize the Widmark equation
- B. Understand retrograde extrapolation for ethanol in various specimens, and the relationship to breath alcohol

2 Training Outline

2.1 Lesson Plan

- A. Widmark’s equation
 1. Estimation of standard drinks in body or number of drinks consumed over a period of time
 2. Limitations of Widmark (e.g. estimation of volume of distribution)
 3. Calculations based on known facts, or reasonable assumptions if facts are unknown
- B. Retrograde extrapolation
 1. Calculation
 2. Range of elimination rates in population

2.2 Required Readings

- A. Levine, Barry, ed. Principles of Forensic Toxicology. 4th ed. Revised. Washington, D.C.: American Association of Clinical Chemistry. 2013. Chapter 13 – Alcohol
Review section on Widmark Equation, pp. 217-218
- B. Harding P., Alcohol Toxicology for Prosecutors: Targeting Hardcore Impaired Drivers. Alexandria, VA: American Prosecutors Research Institute; 2003. Interpretation of alcohol results; pp. 23-40



- C. Garriott, James C. ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 3 – Disposition and Fate of Ethanol in the Body, pp. 86-110
- D. Cowan, Mack. "Retrograde Extrapolation". PowerPoint presentation (2014)

3 Practice

3.1 Safety

None

3.2 Independent Exercises

- A. The trainee will create summary notes over information in the required readings
- B. The trainee will be given specific details of incidents, along with analytical results from an alcohol analysis performed following each incident. The trainee will be asked to perform retrograde extrapolation and/or Widmark calculations for each incident:
 - 1. Estimate the number of drinks the subject consumed.
 - 2. Determine the estimated alcohol concentration (if possible) at a specified time.
 - 3. Describe the symptoms the subject may be exhibiting.
 - 4. Draw illustrative graphs.
 - 5. Explain any assumptions.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-07 SAMPLE PREPARATION FOR ANALYSIS

Duration 1 week

Purpose To familiarize the trainee with an evidence analysis scheme used within the DPS Toxicology (Alcohol/Volatiles) discipline that includes initial examination, sample preparation, volume estimation, and evidence storage.

Prerequisite BA-TM-01-04, BA-TM-01-05, BA-TM-01-06

Note Module can be completed concurrently with BA-TM-01-08, at trainer's discretion.

1 Objectives

1.1 Theoretical

The cases for Toxicology (Alcohol/Volatiles) analysis can be analyzed using this scheme: sample preparation followed by an instrumental analysis via headspace gas chromatography, capable of providing a definitive identification.

By utilizing a consistent flow scheme in evidence examination, an analyst can ensure maximum efficiency and avoid the possibility of cross-contamination. An established scheme can also help to avoid mistakes and will allow the analyst to be able to describe these efforts when testifying.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Understand proper storage of a specimen.
- B. Describe an analysis scheme designed to prevent cross contamination of evidence both within a case and from other cases.
- C. Explain the flow of evidence from the perspective of the Toxicology (Alcohol/Volatiles) section including when and where barcoding will be used.
- D. Discuss the steps taken to prepare a batch using the appropriate software and/or LIMS including creating a worklist, creating a sequence file, and printing labels
- E. Discuss identifying marks on interior and exterior packaging.
- F. Discuss seals before, during, and after analysis.
- G. Know the accepted specimen types for DPS analysis and preferred selection order.
- H. Discuss proper sampling techniques.
- I. Discuss sample volume estimation techniques.
- J. Discuss sample preparation and methodology.
- K. Barcode evidence and folders (as appropriate).
- L. Open and re-seal all containers with correct identifying marks placed on all seals.
- M. Ensure evidence packaging meets shipping requirements for return.
- N. Handle/analyze evidence without contaminating it.
- O. Determine how the evidence will be sampled.



2 Training Outline

2.1 Lesson Plan

The trainee will observe an experienced analyst prepare samples for analysis. During the observation, the experienced analyst will:

- A. Demonstrate the workflow preparation detailed in LIMS-BA-01, including specimen documentation utilizing electronic worksheet or batch worklist.
- B. Determine correct specimen to sample (when multiple submitted)
- C. Handle evidence and packaging.
- D. Correctly mark external and internal packaging.
- E. Sample from specimen container.
- F. Demonstrate techniques used to prevent cross contamination.
- G. Estimate sample volume and note differences in specimen containers.

2.2 Required Readings

- A. LIMS Manual, LIMS-BA-01 – Blood Alcohol Workflow, sections 1 - 4.3
- B. Crime Laboratory Service Manual (CLS),
 1. Evidence and Database Sample Integrity
 2. Evidence Processing
 3. Return of Evidence
- C. Toxicology (Alcohol/Volatiles) Standard Operating Procedure, BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis,
 1. Section 6.1 – Evidence Tracking
 2. Section 6.2 – Evidence Examination
- D. Garriott, James C. ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 10 – Collection and Storage of Specimens for Alcohol Testing

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).

3.2 Equipment

- Hamilton Microlab®
- Basic Equipment

3.3 Observed Performance

- A. The trainer will review all aspects of case handling (barcoding, sampling, marking, sealing, worksheet, and/or worklist completion) with the trainee.
- B. Trainee will observe Toxicology (Alcohol/Volatiles) casework.



3.4 Independent Exercises

The trainee will provide a detailed summary of the analysis scheme. This will include the appropriate software/LIMS workflow and the specific steps that are taken to maintain the chain of custody, prevent cross contamination, and ensure the integrity of the results.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-08 EQUIPMENT

Duration 1 week

Purpose To familiarize the trainee with equipment available in the laboratory for Toxicology (Alcohol/Volatiles) analysis and to enable trainee to safely operate, clean, and maintain laboratory equipment.

Prerequisite BA-TM-01-07

Note Module can be completed concurrently with BA-TM-01-07, at trainer's discretion.
Log books, verifications, and validations can be either physical or electronic.

1 Objectives

1.1 Theoretical

The proper use and maintenance of laboratory equipment is essential to good laboratory practice. All laboratory equipment must be cleaned, verified and used correctly to ensure its reliability. Proper documentation of equipment calibrations and maintenance is crucial to demonstrating quality control in the laboratory. Maintenance and calibration guidelines must be written and well understood by all users.

The proper operation of equipment used for Toxicology (Alcohol/Volatiles) analysis is a necessity to enable the analyst to obtain accurate results when analyzing body fluids for alcohol and other volatiles and to determine the blood alcohol concentration. Understanding the underlying principles is important to enable recognition and resolution of analytical problems.

1.2 Practical

During training, the trainee will:

- A. Become adept at using a laboratory balance and understand the differences between various types.
- B. Learn the attributes, applications, and limitations of the types of pipettes available for use.
- C. Learn how to use and maintain gas generators (as applicable).
- D. Be taught when and how to log maintenance and repair of significant laboratory equipment and know which equipment is considered significant.

Following the completion of this module, the trainee will be able to:

- A. Use a laboratory balance properly.
- E. Weigh varying quantities of substances, demonstrate proper cleaning techniques for spilled substances, and perform quality checks on balances found in the laboratory.
- F. Use proper techniques and understand the purpose, specifications, advantages, and disadvantages of various volume dispensing devices, including different types of pipettes and the Hamilton Microlab® (or equivalent) diluter/dispenser.
- G. Program the diluter/dispenser.
- H. Perform minor repair and/or adjustment on pipettes or diluter/dispenser according to protocol.
- I. Assess accuracy and precision of balance, pipettes, and diluter/dispenser.
- J. Demonstrate proper technique in the use of hotplates/magnetic stirrers and mixers.



- K. Complete relevant documentation pertaining to equipment use, quality control checks and performance verification.
- L. Demonstrate proper use of and differences between the fume/exhaust hoods and biological safety cabinets (laminar flow hoods) found within the laboratory.
- M. Properly monitor temperature using the appropriate device.
- N. Properly use and maintain the refrigerators and freezers. Know what types of substances to store in each.
- O. Properly package items for autoclaving. Load, operate, empty and clean the autoclave, if applicable.
- P. Use a gas generator, including hydrogen, air, and/or nitrogen, and appropriate maintenance that should be performed, if applicable.

2 Training Outline

2.1 Lesson Plan

- A. Introduction to reading and reference materials
- B. Orientation of trainee to the location in the laboratory of available equipment, along with equipment maintenance and verification log book(s) (or electronic equivalent). Identify the significant equipment used in Toxicology (Alcohol/Volatiles) testing.
- C. Balances
 - 1. Differences between various types of balances in the laboratory
 - 2. Instruction on operation and cleaning
 - 3. QC specifications, verification and documentation
- D. Pipettes
 - 1. Disposable
 - a) *Plastic disposable pipettes should be disposed of in trash or as biological waste, as appropriate.*
 - b) *Glass disposable pipettes should be disposed of in glass waste receptacle or biological sharps container, as appropriate.*
 - 2. Glass
 - a) *Maintenance and cleaning*
 - b) *QC specifications, calibration and documentation*
 - 3. Air displacement
 - a) *Instruction on choice of size and operation of different pipettes*
 - b) *Maintenance and cleaning*
 - c) *QC specifications, calibration and documentation*
 - 4. Positive displacement
 - a) *Instruction on choice of size and operation of different pipettes*
 - b) *Maintenance and cleaning*



- c) *QC specifications, calibration and documentation*
 - 5. Repeater
 - a) *Instruction on choice of size and operation of different pipettes*
 - b) *Maintenance and cleaning*
 - c) *QC specifications, calibration and documentation*
 - 6. Diluter/dispenser
 - a) *Programming*
 - b) *Maintenance and cleaning*
 - c) *QC specifications, calibration, and documentation*
 - 7. Other types of volume dispensing devices (e.g. bottletop dispensers)
- E. Hoods (2 types)
 - 1. Chemical Fume Hoods
 - a) *Principles of operation*
 - b) *Uses of fume hoods*
 - c) *Safety checks and maintenance*
 - 2. Laminar Flow Biological Safety Cabinet (Biological Hood)
 - a) *Principles of operation*
 - b) *Uses of laminar flow hoods*
 - c) *Certification and maintenance*
- F. Autoclave (if applicable)
 - 1. Instructions on usage applications (sterilization of solutions, biological waste, proper labeling and disposal of biological waste, etc.), operation and cleaning
 - 2. Logbook recording sterilization of biological waste
- G. Refrigerators and/or Freezers
 - 1. Location and storage application
 - 2. Temperature specifications and alarm device
 - 3. Cleaning (routine and major)
 - 4. QC documentation
- H. Thermometers (standard and digital)
 - 1. Instructions on handling, reading, and maintenance
 - 2. QC specifications, calibration and documentation
 - 3. NIST traceable documentation
- I. Vortexers, rotators, and/or magnetic stirrers (if applicable)
 - 1. Instructions on use
 - 2. Cleaning and maintenance



- J. Laboratory glassware (e.g., volumetric flasks, graduated cylinders, tissue grinders, etc.)
 - 1. Class A glassware
 - 2. Cleaning and maintenance
 - 3. Proper disposal of broken glassware
- K. Gas generators (if applicable)
 - 1. Instructions on use
 - 2. Maintenance

2.2 Required Readings

- A. Crime Laboratory Service Manual – Laboratory Equipment chapter,
 - 1. General Requirements section
 - 2. Significant Equipment section
 - 3. Laboratory Software section
 - 4. Calibration Program section
- B. Toxicology (Alcohol/Volatiles) Standard Operating Procedures,
 - 1. BA-01-02 – Equipment
 - 2. BA-02-03 – Instructions for Using the Hamilton Microlab®
- C. User manuals for specific laboratory equipment available (pipettes, balances, centrifuges, autoclaves, laminar hoods, fumes hoods, etc.)
- D. Laboratory equipment maintenance and calibration log book(s) or equivalent
- E. Local laboratory policy and/or form for balance verifications/checks

2.3 Suggested Readings

The following are available in the Laboratory Resources/E-Train Collection on SharePoint at the QA site.

- A. “A Guide to Pipetting”
- B. “A Guide to Laboratory Balances”

3 Practice

3.1 Safety

- A. Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).
- B. Use extreme caution when operating an autoclave. Do not open autoclave until chamber pressure reads zero and it has cooled to safe handling temperature.
- C. Handle thermometers carefully.
Note: Mercury is a chemical hazard and should be handled as such whenever a thermometer is broken and mercury is released.



3.2 Equipment

- Laboratory balance
- Diluter/dispenser
- Pipettes
- Autoclave (if applicable)
- Thermometer
- Laboratory glassware
- Gas generator (if applicable)

3.3 Observed Performance

- A. Trainer will demonstrate to the trainee proper technique for operating laboratory equipment.
- B. Trainer will give instructions on documentation of quality control procedures and calibration of pertinent equipment.
- C. Trainer will review with the trainee a calibration certificate from the service technician for a balance and a pipette.

3.4 Independent Exercises

Note: For exercises A and B, a balance with at least 3 decimal places will be used.

A. Balances:

1. Use of each type of balance in the laboratory will be demonstrated.
2. The trainee will use NIST traceable weights to check calibration of a balance, e.g. 200 mg weight to assess weight of dispensed 200 μ L of water from a calibrated positive displacement pipette.

B. Pipettes:

1. Prior to analytical work, the trainee will read the instructions and become familiar with each type of pipette in use.
2. Using each type of pipette subject to verification (BA-01-02), including diluter/dispenser, the trainee will practice dispensing water to a balance and comparing its weight to the expected weight. Repetition will be performed until the trainee is satisfied with their technique.

- C. **Autoclave:** The trainee will practice autoclaving biological waste under the supervision of the trainer (if applicable).

4 Assessment

4.1 Competency and Qualifying Examination

- A. A pipette competency test will be given to demonstrate skill in using diluter/dispenser and various mechanical pipettes commonly used in Toxicology (Alcohol/Volatiles) analysis.
 1. The trainer will observe and record weights as the trainee performs the test by dispensing specific amounts of water onto the balance.
 2. The trainer will assess the trainee's ability to use each type of pipette utilized in analysis and to pipette with accuracy and precision.



- B. A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-09 FUNDAMENTAL OPERATIONS

Duration 1 to 2 weeks

Purpose To familiarize the trainee with the fundamental operations for Toxicology (Alcohol/Volatiles) analysis

This module includes solution preparation and basic instrument operation.

Prerequisite BA-TM-01-08

1 Objectives

1.1 Theoretical

The proper use of the fundamental tools used for Toxicology (Alcohol/Volatiles) analysis (solutions, gas chromatographs, and their controlling software) is a necessity to enable the analyst to obtain accurate results when analyzing body fluids for the presence of alcohol and other volatiles and to quantify the amount of alcohol present.

Understanding the underlying principles is important to enable recognition and resolution of analytical problems.

1.2 Practical

During training, the trainee will become adept at the preparation of stock solutions. The trainee will perform gas chromatography instrument software program functions and basic instrument operation and maintenance.

Following the completion of this module, the trainee will be able to:

- A. Use pipettes and volumetric glassware for the preparation of reagents used in the analysis of alcohol and other volatiles.
- B. Label reagents properly.
- C. Set up the gas chromatograph for analysis and run samples.
- D. Perform basic gas chromatograph instrument maintenance.
- E. Understand/Perform instrument software program functions, including instrument control, autosampler control, sequence files, calibration, batch run, and analytical report generation.

2 Training Outline

2.1 Lesson Plan

- A. Stock solutions
 1. Purpose
 2. Preparation
 3. Storage and expiration
 4. Labeling
- B. Purchased solutions
 1. Purpose
 2. Storage and expiration



C. Gas Chromatograph basics

1. Software
2. Hardware
3. Operation
4. Maintenance

2.2 Required Readings

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedures,
1. BA-01-01 – Chemicals, Reagents and Standards
 2. BA-03-01 – Preparation of NaCl/n-Propanol Internal Standard Solution
 3. BA-03-02 – Preparation of Volatile Mixture Standard
- B. Crime Laboratory Service Manual – Laboratory Equipment chapter,
1. Laboratory-Prepared Reagents section
 2. Commercially-Prepared Reagents section
- C. Literature pertaining to instrumentation/software utilized in operating laboratory.
- D. Garriott, James C., ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 8 – Methods for Fluid Analysis, *Sections 8.4 - 8.6, pp. 258-264*
- E. Moffat, Anthony C., Osselton, M. David, Widdop, Brian, Watts, Jo, eds. Clarke's Analysis of Drugs and Poisons, 4th ed. Gurnee, IL: Pharmaceutical Press, 2011. Chapter 14 – Volatile Substances.
- F. Karch, Steven B., ed. Drug Abuse Handbook, 2nd ed. Boca Raton, FL: CRC Press, 2007. *Section 5.2.3.3 – Gas Chromatographic Methods, pp. 341- 345.*

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).

3.2 Standards, Controls, Reagent Preparation

- Purified water
- Ethanol standard solutions (NIST traceable)
- Reagents listed in Toxicology (Alcohol/Volatiles) Standard Operating Procedures,
 - BA-03-01 – Preparation of NaCl/n-Propanol Internal Standard Solution
 - BA-03-02 – Preparation of Volatile Mixture Standard

3.3 Equipment

- Gas chromatographic system with headspace autosampler
- Pipettes and/or automatic diluter/dispenser
- Glassware



3.4 Observed Performance

The trainer will:

- A. Demonstrate process for setting up the instrumentation for analysis.
- B. Demonstrate process for setting up a calibration and batch analysis.
- C. Demonstrate capabilities of instrument software and functions available.
- D. Discuss and/or demonstrate routine instrument maintenance required.

3.5 Supervised Performance

The trainee will:

- A. Demonstrate instrument set up to trainer.
- B. Observe and assist in demonstration of routine maintenance performed.
- C. Demonstrate knowledge of software capabilities.
- D. Prepare stock solutions of NaCl/n-propanol internal standard and volatile standard mix, and label each appropriately.

3.6 Independent Exercises

For each exercise below, the trainee will prepare a sequence file for the gas chromatograph, perform the analysis, and prepare the batch results summary.

The sampling techniques used in the exercises should reflect those that are used during casework unless otherwise specified.

- A. **Sampling Precision** – The trainee will perform a reproducibility study to demonstrate precision when sampling.
 1. The trainee will prepare and perform GC analysis of replicate samples (at least 10) of one ethanol standard to evaluate sampling reproducibility. The trainee will prepare a spreadsheet calculation of the percent relative standard deviation (RSD) of the area measurements for the internal standard and ethanol.
 2. Evaluation Criteria: Demonstrating a %RSD less than 5% is satisfactory for reproducibility; the trainee may then progress to other exercises.
- B. **Sampling Carryover** – The trainee will perform an exercise to evaluate ethanol carryover from sampling.
 1. The trainee will prepare sample vials with a high concentration ethanol sample (such as 0.500 g/100 mL) and at least five negative samples. The trainee will first sample the high concentration followed by the negative samples **without** rinsing in between samples. The vials are run on the gas chromatograph in the order in which they were sampled. From this the trainee can judge the number of rinses required to avoid carryover of ethanol, when using that sampling device.
 2. Evaluation Criteria: The number of blanks run before the first blank with no detectable ethanol is equal to the number of rinses needed to avoid carryover.



C. Retention Time

1. The trainee will prepare the volatiles standard mix solution and the individual volatile standard solutions and perform gas chromatography to identify each component of the mix. The trainee will prepare a reference chart to document the individual retention time for each volatile component on each analytical column.
2. The trainer will demonstrate how retention times are updated in software and discuss identification criteria from SOP.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Each exercise should include a summary sheet with any conclusions and necessary calculations.
- C. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-10 HEADSPACE GAS CHROMATOGRAPHY

Duration 2 to 3 weeks

Purpose To familiarize the trainee with the theory and the validation of headspace gas chromatography analysis.

Prerequisite BA-TM-01-09

Objectives

1.1 Theoretical

Gas chromatography is used in many types of scientific analysis. Headspace gas chromatography is the technique of choice for the quantitative analysis of ethanol in biological specimens. Understanding the principles which govern headspace gas chromatography analysis is vital in obtaining an overall understanding of the analysis of alcohol and other volatiles.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Explain the theory and applications of gas chromatography.
- B. Explain Henry's Law.
- C. Determine the limits of detection of the gas chromatograph and/or instrumental method.
- D. Document requirements listed in Toxicology (Alcohol/Volatiles) SOP pertaining to instrumentation.
- E. Evaluate method parameters to produce good chromatographic results.
- F. List components utilized in instrumentation (column type, detector type, various gases, etc.).
- G. Set up instrumentation for analysis and perform instrument validation measures
- H. Explain instrument response in relation to autosampler heating temperature and time.
- I. Review instrument capabilities and reinforce proficiency in operating the instrument software.
- J. Demonstrate skills of preparing and analyzing calibrators and samples, including constructing a calibration curve.

2 Training Outline

2.1 Lesson Plan

- A. Headspace Gas Chromatograph
 1. Theory
 - a) *Advantages*
 - b) *Disadvantages*
 - c) *Henry's Law*
 2. Operation
 - a) *Detector*



- b) *Column (specified in SOP)*
 - i. *Composition*
 - ii. *Range of temperature*
 - iii. *Quantitative versus qualitative*
 - c) *Gases*
 - i. *Van Deemter curve*
 - ii. *Carrier gas*
 - iii. *Detector/Auxiliary gas*
 - d) *Optimization*
3. Calibration
- a) *Single point vs multipoint*
 - b) *Weighting*
 - c) *Manual calculations*

2.2 Required Readings

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedure, BA-02-04 – Validation of Significant Equipment
- B. Moffat, Anthony C., Osselton, M. David, Widdop, Brian, Watts, Jo, eds. Clarke's Analysis of Drugs and Poisons, 4th ed. Gurnee, IL: Pharmaceutical Press, 2011. Chapter 40 – Gas Chromatography, pp. 636-654
- C. "A Technical Guide for Static Headspace Analysis Using GC." Restek
- D. Christmore et. al, "Improved Recovery and Stability of Ethanol in Automated Headspace Analysis." *Journal of Forensic Sciences*, 29.4 (1984): pp. 1038-1044
- E. Local documents and/or instructions pertaining to instrumentation/software utilized in operating laboratory
- F. Flanagan, R.J. et al, Fundamentals of Analytical Toxicology. John Wiley & Sons, Ltd., 2007. *Section 14.2.4 – Calibration Graphs*, pp. 365-375

2.3 Suggested Readings

- A. "DPS Method Validation for Blood Alcohol Analysis Utilizing Headspace Gas Chromatography" (1978)
- B. DPS method validation "Multipoint Curve Quantitation Method for Alcohol Analysis" (Valid-Method-SYS-BA-MultipointQuant-2016-0531)

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).



3.2 Standards, Controls, Reagent Preparation

- A. Purified water
- B. Ethanol standard solutions (NIST traceable)
- C. NaCl/n-Propanol internal standard solution
- D. Volatile mix standard

3.3 Equipment

- A. Gas chromatograph with dual flame ionization detectors
- B. Two different chromatographic columns suitable for alcohol analysis
 1. Quantitative column (BAC-1 or comparable)
 2. Qualitative column (BAC-2 or comparable)
- C. Headspace autosampler
- D. Crimper
- E. Vials, caps, and stoppers
- F. Positive displacement or air displacement pipette and tips, and/or automatic diluter/dispenser

3.4 Observed Performance

Trainer will discuss theoretical and practical objectives with the trainee. It is recommended that this discussion be completed after the independent exercises.

3.5 Independent Exercises

Note: All samples are prepared along with 1400 μ L of NaCl/n-propanol internal standard solution, unless otherwise noted.

A. Calibration

1. The trainee will prepare a set of calibrators and all other standards required for alcohol batch analysis, per the SOP. The trainee will set up the instrument for a calibration and sequence run, construct a calibration curve, and evaluate the analyzed standards.
2. Evaluation Criteria: The resulting calibration curve and standards must meet all criteria as outlined in the SOP (BA-02-01).

B. Thermostat - This study will show the optimum operating parameters for the autosampler.

Note: This study is optional and may be satisfied by discussion with trainer.

1. Prepare ethanol samples and analyze at various temperatures and heating times. Headspace vials should be prepared at the following concentrations:
 - a) Ethanol at 0.080 g/100mL
 - b) Ethanol at 0.50 g/100mL
 - c) (optional) 200 μ L purified water
2. Evaluation Criteria: Plot result values (area counts) for ethanol and n-propanol at chamber temperature versus heating time to identify optimum response.



- C. **Limit of Detection** - The LOD is administratively defined as 0.005 g/100mL.
1. Prepare at least three 0.005 g/100 mL ethanol samples and analyze. Alternatively, the trainee may serial dilute a 0.080 g/100 mL ethanol standard solution to at least 0.005 g/100 mL, then prepare and analyze at least one vial of each dilution level.
 2. Evaluation Criteria: The signal-to-noise ratio for the ethanol peak must be greater than 3:1. If performing serial dilution, the LOD is the smallest concentration of ethanol at which criteria is met.
- D. **Testing Carryover** - This study will prove that the testing instrument will not produce a detectable amount of carryover when analyzing a sample that is twice the amount of the highest calibrator.

Note: A 0.50 g/100 mL ethanol standard solution may be substituted for the 1.0 g/100 mL standard at trainer's discretion.

1. Sample and analyze (200 µL purified water or ethanol standard solution and 1400 µL **purified water**) in the following order:
 - a) *Blank*
 - b) *0.001 g/100mL*
 - c) *Blank*
 - d) *1.0 g/100mL*
 - e) *Blank*
2. Evaluation Criteria: The ethanol peak area in the blank succeeding the 1.0 g/100mL solution must not exceed the peak area for ethanol in the 0.001 g/100mL solution. Thresholds may need to be lowered in order to determine peak area.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Each exercise should include a summary sheet with any conclusions and necessary calculations.
- C. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-11 ANALYTICAL PROCEDURES

Duration 1 month

Purpose To familiarize the trainee with procedures necessary for the analysis of specimens for the presence of ethanol and other volatile compounds.

Prerequisite BA-TM-01-10

1 Objectives

1.1 Theoretical

Following the approved analytical procedures ensures that an accurate alcohol concentration can be determined. Concentration levels can vary based on the type of sample specimen and understanding these concentration differences is important when interpreting results.

1.2 Practical

During training, the trainee will gain understanding of the analysis process, including the evaluation of specimens, biological sample preparation, and assessment of quality control data. The trainee will learn which samples DPS deems acceptable for analysis, and be able to correlate the results from different specimen sources.

Following the completion of this module, the trainee will be able to:

- A. Record observations of samples.
- B. Document the condition of evidence.
- C. Complete a Toxicology (Alcohol/Volatiles) Analysis Worksheet (LAB-BA-01 or electronic equivalent) for each training sample.
- D. Describe how to process clotted samples using a tissue grinder.
- E. Understand sample factors affecting results.

2 Training Outline

2.1 Lesson Plan

- A. Reagent preparation
 1. Review of reagent preparation
 2. Purpose for each reagent
 3. Stability of standards
 4. Chemical hazard awareness/waste disposal review
- B. Materials
 1. Sample vial racks
 2. Sample vials, caps, and stoppers
 3. Reagents
 4. Laminar flow hood
- C. Sample preparation
 1. Sample hazard awareness/waste disposal



2. Steps of procedure
 3. Clots and use of tissue grinder
 4. Matrix (sample origin) effects
- D. Evaluation of results
1. Physiology review and how type of sample affects results
 2. Microbial effects/preservatives/anticoagulants
 3. Sample age
 4. Criteria for acceptability for reporting results

2.2 Required Readings

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedures, BA-02-01 – Toxicology (Alcohol/Volatiles) Analysis
- B. Laboratory Information Management System Instructions, LIMS-BA-01 – Blood Alcohol Workflow, *review sections 1 - 4.3*
- C. Garriott, James C. ed. Medicolegal Aspects of Alcohol. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 9 – Quality Assurance
- D. Amick, G.D. and Habben, K.H., “Inhibition of Ethanol Production by *Saccharomyces cerevisiae* in Human Blood by Sodium Fluoride”, *Journal of Forensic Sciences*, 42.4 (1997): pp. 690-2
- E. Chang, J. and Kollman, S.E., “The Effect of Temperature on the Formation of Ethanol by *Candida Albicans* in Blood”, *Journal of Forensic Sciences*, 34.1 (1989): pp. 105-109
- F. Lough, P.S. and Fehn, R. “Efficacy of 1% Sodium Fluoride as a Preservative in Urine Samples Containing Glucose and *Candida albicans*”, *Journal of Forensic Sciences*, 38.2 (1993): pp. 266-271
- G. Blackmore, D.J., “The Bacterial Production of Ethyl Alcohol”, *Science and Justice*, 8.2 (1968): pp. 73-8
- H. Jones, A.W. “Salting Out Effect of Sodium Fluoride and Its Influence on the Analysis of Ethanol by Headspace Gas Chromatography”, *Journal of Analytical Toxicology*, 18.5 (1994): pp. 292
- I. Tiscione, N. “The Impact of Hemolysis on the Accuracy of Ethanol Determinations.” *Journal of Analytical Toxicology*, Vol 39, Issue 8 (2015): pp.672-673
- J. Senkowski, C.M. and Thompson, K.A. “The Accuracy of Blood Alcohol Analysis Using Headspace Gas Chromatography When Performed on Clotted Samples.” *Journal of Forensic Sciences*, Vol 35, No 1 (1990): pp. 176-180

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).



3.2 Standards, Controls, Reagent Preparation

- Ethanol standard solutions (NIST traceable)
- NaCl/n-propanol internal standard
- Volatile mixture standard

3.3 Equipment

- Sample vial racks
- Laminar flow hood
- Sample vials
- Sample vial caps
- Pipettes and/or automatic diluter/dispenser
- Crimper for sample vials
- Gas chromatographic system with headspace autosampler

3.4 Observed Performance

- A. The trainer will review sample preparation and batch creation. The trainer will also discuss any documentation and safety issues.
- B. The trainer will demonstrate or discuss how to use the tissue grinder, including how to properly decant liquids. Appropriate cleaning technique will also be shown.

3.5 Supervised Performance

The trainee will complete analysis of at least 10 unknown biological samples, including at least one blood and one urine sample. The trainee will complete a LAB-BA-01 (or electronic equivalent) for each sample, enter all required information for the run into the gas chromatograph software, perform sample preparation and analysis, and prepare a batch results summary sheet.

- A. The batch must include calibration of the instrument as well as all calibration check and control samples required in casework.
- B. The unknown samples may be obtained from the following sources:
 1. Samples prepared and tested by the trainer (or other trained laboratory personnel).
 2. Proficiency samples for which the manufacturer has supplied final summary information.

Note: *The results from the trainee's analysis must correspond to the results of a trainer's reanalysis of the samples or the original results within an expected allowance for evaporation.*

3.6 Independent Exercises

Matrix Conversions

- A. The trainer and trainee will discuss the expected concentration differences between different specimens.
- B. Following the discussion, the trainee will be given data from several specimen matrices from the same person and asked to calculate the ethanol concentration difference from one specimen type to another. This may be data from a real case or a hypothetical situation created by the trainer.



4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-12 MEASUREMENT UNCERTAINTY

Duration 1 to 2 weeks

Purpose To familiarize the trainee with measurement uncertainty estimate associated with reporting alcohol content.

Prerequisite GLT-TM-UNC

1 Objectives

1.1 Theoretical

Measurement uncertainty takes into account the accuracy and precision of the measurement and provides a range of values that could reasonably include the true measurement.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Understand common terms associated with measurement uncertainty.
- B. Identify and explain measurement uncertainty components of the reported alcohol uncertainty estimate.

2 Training Outline

2.1 Lesson Plan

- A. Terminology
 1. Normal Distribution
 2. Rectangular Distribution
- B. Identifying and evaluating uncertainty components
- C. Calculating an uncertainty budget

2.2 Required Readings

- A. Toxicology (Alcohol/Volatiles) Standard Operating Procedure, BA-02-02 – Measurement Uncertainty Estimate
- B. Toxicology (Alcohol/Volatiles) Training Manual, BA-TM-03-01 – Preparation of Ethanol Solutions
- C. Current Texas DPS Alcohol Uncertainty Estimate
- D. “DPS Blood Alcohol Matrix Effect Study” ([Valid-Method-AUS-TOX-BAMatrixEffect-2016-1005](#))
- E. Garriott, James C. ed. *Medicolegal Aspects of Alcohol*. 6th ed. Tucson, AZ: Lawyers & Judges Publishing Company, Inc., 2015. Chapter 19 – Uncertainty in Blood and Breath Alcohol Measurements.
- F. It is recommended that the trainee complete online training: RTI International's Center for Forensic Sciences Continuing Education Program, *Introduction to Uncertainty in Forensic Chemistry and Toxicology 1, 2, & 3*. Alternatively, trainee can independently review handouts from the course and discuss concepts with trainer.

2.3 Suggested Readings

Bell, Stephanie, “A Beginner’s Guide to Uncertainty of Measurement”, Measurement Good Practice Guide No. 11, Issue 2, National Physical Laboratory. (1999)



3 Practice

3.1 Safety

Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).

3.2 Standards, Controls, Reagent Preparation

- A. Purified water
- B. Ethanol standard solutions (NIST traceable)
- C. NaCl/n-Propanol internal standard solution

3.3 Equipment

- A. Gas chromatograph with dual flame ionization detectors
- B. Two different chromatographic columns suitable for alcohol analysis
 1. Quantitative column (BAC-1 or comparable)
 2. Qualitative column (BAC-2 or comparable)
- C. Headspace autosampler
- D. Crimper
- E. Vials, caps, and stoppers
- F. Positive displacement or air displacement pipette and tips, or automatic diluter/dispenser

3.4 Independent Exercises

Note: All samples are prepared along with 1400 μ L of NaCl/n-propanol internal standard solution. A calibration will be performed prior to analysis.

- A. **Uncertainty Due to Reproducibility** - This study will demonstrate how reproducibility contributes to the measurement uncertainty estimate by comparing sample preparation techniques.
 1. Using a purchased or prepared ethanol standard:
 - a) *Prepare and analyze ten headspace vials using the automatic diluter/dispenser.*
 - b) *Prepare and analyze ten headspace vials using multiple pipettes.*
 2. Evaluation: The trainee will prepare a spreadsheet calculation of the percent relative standard deviation (RSD) of the area measurements for the internal standard and ethanol. The results from each sampling technique will be compared.

Note: If the training lab does not have pipettes sufficient to pipet the required quantities, separate methods for pipetting the sample (200 μ L) and the internal standard (1400 μ L) may be used.
- B. **Uncertainty Due to Standards** - This study will demonstrate how the source of ethanol standards contributes to the measurement uncertainty estimate.
 1. Using a prepared ethanol solution and purchased ethanol standard of the same concentration:
 - a) *Prepare and analyze ten headspace vials of the prepared ethanol solution.*
 - b) *Prepare and analyze ten headspace vials of the purchased ethanol standard.*



2. **Evaluation:** The trainee will prepare a spreadsheet calculation of the average concentration for each ethanol solution and their percent difference from the expected concentration. The results from each source will be compared.

Note: It is recommended the solutions used be a concentration equal to one of the controls used in alcohol analysis.

4 Assessment

4.1 Competency and Qualifying Examination

A training module review quiz is required.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Each exercise should include a summary sheet with any conclusions and necessary calculations.
- C. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-13 REPORT WRITING

Duration 2 to 5 days

Purpose To familiarize and provide the trainee with the process and guidelines for Toxicology (Alcohol/Volatiles) casework report writing and for technical and administrative case reviews.

Prerequisite BA-TM-01-11

1 Objectives

1.1 Theoretical

The laboratory report is used to communicate analytical results to its reader. The report should be correct in all details including description of evidence, results of analysis, and any administrative information available to the laboratory.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Explain all the steps necessary in producing a case report after the completion of the analysis of a case.
- B. Explain the procedure for getting the report to the officer or other party.
- C. Enter the results from case analysis into the laboratory reporting software to produce a report.
- D. Determine which notes are appropriate for use in a report.
- E. Technically review a completed case record.
- F. Administratively review a completed case record.

2 Training Outline

2.1 Lesson Plan

- A. Case Documentation
 1. Documentation practices
 2. Case related correspondence
- B. Reporting Guidelines
 1. Toxicology (Alcohol/Volatiles) results
 - a) *Review of reporting units*
 - b) *Selection of proper value for report*
 - c) *Assurance of ethanol results (quantitative agreement from two independent analyses, acceptable chromatography, and retention time match)*
 - d) *Detection of other volatiles*
 - e) *Result notes*
 - f) *90 codes*
 2. Qualitative results
 3. Quantitative results



C. Reporting

1. Elements of report
2. Format of report
3. Types of reports
4. Report distribution
5. Disposition of evidence
6. Re-examination
7. Using laboratory reporting software

D. Case Review

1. Technical review
2. Administrative review

2.2 Required Readings

A. Toxicology (Alcohol/Volatiles) Standard Operating Procedures

1. BA-01-03 – Disposition of Toxicology Evidence
2. BA-01-04 – Case Documentation
3. BA-01-07 – Guidelines for Case Review

B. Laboratory Information Management System Instructions,

1. LIMS-BA-01 – Blood Alcohol Workflow, *sections 4.4 - 5*
2. LIMS-BA-02 – Amended Alcohol Analysis
3. LIMS-BA-03 – Alcohol Analysis 90 Codes

C. Crime Laboratory Service Manual,

1. Laboratory Service Requests, *Requests for Reanalysis of Evidence section only*
2. Laboratory Records
3. Laboratory Reports, Letters, and Certificates
4. Review of Laboratory Records

3 Practice

3.1 Safety

None

3.2 Observed Performance

- A. The trainer will demonstrate batch reporting, including uploading of results into LIMS, manual entry of results into LIMS, and entry of result and/or disposition notes. At least ten cases must be demonstrated.
- B. The trainer will demonstrate review of batch data and technical and administrative review of cases. At least ten cases must be demonstrated.



3.3 Supervised Performance

The trainee will perform technical and administrative review on at least 10 cases. The trainer will then review the cases and discuss any findings with the trainee.

Note: *Trainee can go through review process in LIMS under trainer's supervision, but **will not** complete milestones.*

3.4 Independent Exercises

Using the data obtained from the Supervised Performance exercise in BA-TM-01-11 (Analytical Procedures) the trainee will evaluate and report the results of at least one sample in LIMS as a test case.

4 Assessment

4.1 Competency and Qualifying Examination

The Independent Exercise described above will serve as the competency test in report writing.

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for casework.



BA-TM-01-14 PRACTICAL ASSESSMENT

Duration 1 to 2 weeks

Purpose To assess the trainee's ability to independently analyze, evaluate, and report results for biological samples.

Prerequisite BA-TM-01-13

1 Objectives

1.1 Theoretical

The trainee will be able to independently demonstrate their familiarity with the instrumentation and appropriate software, their competence in the analysis of biological specimens, and their ability to evaluate the analytical results in accordance with laboratory policy.

1.2 Practical

Following the completion of this module, the trainee will be able to:

- A. Prepare biological samples for analysis
- B. Enter all required information for the analysis session into the gas chromatograph software
- C. Perform instrumental analysis of biological samples for alcohol and other volatiles
- D. Evaluate data
- E. Prepare a batch results summary

2 Training Outline

2.1 Lesson Plan

- A. Sample examination and analysis
- B. Data assessment
- C. Computation and documentation of analytical results

2.2 Required Readings

None

3 Practice

3.1 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-01-02 (Safety).

3.2 Standards, Controls, Reagent Preparation

- Ethanol standard solutions (NIST traceable)
- NaCl/n-propanol internal standard
- Volatile mixture standard

3.3 Equipment

- Sample vial racks
- Laminar flow hood



- Sample vials, caps, and stoppers
- Crimper for sample vials
- Gas chromatographic system with headspace autosampler
- Pipettes and/or automatic diluter/dispenser

3.4 Independent Exercise (optional)

The trainee will complete analysis of at least 10 unknown biological samples, including at least one blood and one urine sample. The trainee will complete a LAB-BA-01 (or electronic equivalent) for each sample, enter all required information into the gas chromatograph software, perform sample preparation and analysis, evaluate the analytical data, and prepare a batch results summary sheet.

- A. The batch must include calibration of the instrument as well as all calibration check and control samples required in casework.
- B. The unknown samples may be obtained from the following sources:
 1. Samples prepared and tested by the trainer (or other trained laboratory personnel).
 2. Proficiency samples for which the manufacturer has supplied final summary information.

Note: *The results from the trainee's analysis must correspond to a trainer's reanalysis of the samples or the original results within an expected allowance for evaporation.*

4 Assessment

4.1 Competency and Qualifying Examination

A. Competency

The trainee will independently analyze at least 15 unknown biological samples, including at least one blood and one urine sample. Sources for these samples are outlined in the Independent Exercise. **These samples must be recently analyzed by a qualified analyst.**

1. Results of the trainee's duplicate samples must meet the criteria in BA-02-01, 7.1 Evaluation of Ethanol Results.
2. The trainee's average result must meet the criteria in BA-02-01, 5.2 Ethanol Solutions. The expected value is the recently analyzed average result.
3. At the trainer's discretion:
 - a) *The Competency may substitute for the Independent Exercise, provided the trainee performs the task without any assistance.*
 - b) *Re-analysis or resampling of competency sample(s) may be conducted for instances outside the control of the trainee (e.g. instrument malfunction).*

B. Qualifying Examination

The trainer will administer a comprehensive written examination approved by the Toxicology (Alcohol/Volatiles) advisory board. The score on the written exam must be 75% or better to pass.



C. Mock Trial

The trainee will perform a mock trial using the test case from the Independent Exercise in BA-TM-01-13 (Report Writing).

Note: *It is recommended the mock trial be performed following the written examination, but this will be decided at the trainer's discretion.*

4.2 Evaluation of Training

- A. The trainee and trainer will complete the Toxicology (Alcohol/Volatiles) Training Checklist (LAB-BA-TM-01).
- B. Successful completion of this module is determined by the trainer and is a prerequisite for supervised casework.



02 FORMS

TRAINING FORMS

	Document Name	FRN
1	Toxicology (Alcohol/Volatiles) Training Checklist	LAB-BA-TM-01



03 APPENDIX

BA-TM-03-01 PREPARATION OF ETHANOL SOLUTIONS

1 Scope

Preparation of ethanol standard solutions and ethanol spiked biological samples for use in training.

2 Specification

10% Ethanol Stock Solution; respective Ethanol Solutions

3 Safety

Biological specimens may contain infectious agents. Chemicals used may be toxic and flammable. Use appropriate laboratory safety precautions and observe Universal Bloodborne Pathogens precautions, as outlined in BA-TM-02 (Safety).

4 Equipment and Materials

- Volumetric flasks
- 13.33 mL custom calibrated volumetric pipette
- Volumetric TD (to deliver), positive displacement, or air displacement pipettes
- Balance
- Ethyl alcohol, anhydrous, 200 proof
- 95% Ethanol
- Purified water

5 Instructions

A. 10% Ethanol Stock Solution (10 grams/100 milliliters)

1. Prepare by combining ethanol and purified water in a volumetric flask using the following ratio:
 13.33 mL 95% ethanol (or ~10.526 g by weight) per 100 mL water
2. Or, prepare by weighing 100 grams of 100% (200 proof) ethanol and diluting to 1000 mL with water. Other proportional weights and volumes may be used.

B. Ethanol Solutions

1. Dilute the volume of 10% ethanol stock solution with purified water in a volumetric flask using the ratios in the table:

Ethanol Standard Solution	Amount 10% stock solution per 100 mL water
0.01% (0.010 grams/100 mL)	0.1 mL
0.08% (0.080 grams/100 mL)	0.8 mL
0.10% (0.100 grams/100 mL)	1.0 mL
0.15% (0.150 grams/100 mL)	1.5 mL
0.20% (0.200 grams/100 mL)	2.0 mL



Ethanol Standard Solution	Amount 10% stock solution per 100 mL water
0.30% (0.300 grams/100 mL)	3.0 mL
0.40% (0.400 grams/100 mL)	4.0 mL
0.50% (0.500 grams/100 mL)	5.0 mL

2. Different volumes may be prepared in the same proportions as specified.
3. Additional concentrations of ethanol solutions may be prepared using the appropriate volume of 10% stock solution.
4. Allow all solutions to equilibrate at room temperature before using.

C. Ethanol spiked biological samples

Ethanol spiked biological samples for use in training can be prepared in the same manner as ethanol standard solutions following the directions in section 5.B.

- 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.

Note: Prepared ethanol standard solutions are for use in training only and shall not be used for casework. Storage containers shall be clearly labeled to indicate this.

6 Testing, Storage, Expiration, and Disposal

- A. Testing of solutions shall be performed prior to use by trainee if required in the respective training module.
- B. Store solutions at room temperature or refrigerate. Shelf life is one year.