Department of Forensic Science

TOXICOLOGY TRAINING MANUAL

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Purpose and Scope

1.1.1 The purpose of this manual is to define the training program for forensic lab specialists, forensic scientists, and toxicologists working in the Toxicology section as employees of the Commonwealth of Virginia Department of Forensic Science. This work is intended to be used in a formal training program that will establish a certain minimum standard of professional competency throughout the Toxicology section statewide.

1.1.2 The manual is organized in modules and each module outlines the objectives, methods of instruction, modes of evaluation, and study questions. Additional study questions may be assigned to the trainee by the Training Coordinator (TC), upon approval of the Toxicology Program Manager, based upon laboratory-specific needs and considerations. These study questions, along with acceptable responses, will be provided to the Toxicology Program Manager (PM) for consideration toward inclusion into future revisions of the Toxicology Training Manual.

1.1.3 The training program covers theory and methodology of instrumentation, analytical techniques, interpretation of analytical results, report writing, data and case review, and handling of evidence.

1.1.4 The training program provides exposure to courtroom testimony and legal aspects throughout the training and assists in developing the skills necessary to be an effective expert witness.

1.1.5 The training program evaluates the progress and performance of the trainee within each module. Upon completion of each module, the trainee will give an oral presentation on the module material which will be followed by a question/answer session to ensure the trainee understands the module material.

1.1.6 The sequence in which the modules are presented should not be considered as a mandatory order of instruction.

1.1.7 The trainee will complete a mini-technical examination after the first 8 modules and a second mini-technical final on the remaining modules.

1.1.8 It is recognized that some roles within the Toxicology section may only perform certain analyses or functions. Therefore, the following is a suggested list of modules to be completed by each role. This may be modified by the TC, Section Supervisor, and PM as necessary to meet the goals of the fully trained position.

Module	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Forensic Laboratory Specialist (FLS)	X	X	X	X	X	X	X	X*	X*	X*	X**		X*		
Forensic Scientist (FS)	X	X	X	X	X	X	X	X	X	X	X**	X	X		
Forensic Toxicologist (Tox)	X	X	X	X	X	X	X	X	X	X	X**	X	X	X	X

*Modules may be completed by a Forensic Laboratory Specialist dependent upon their role.

**See Section 1.1.9 for Ethanol Content of Alcoholic Beverages training.

1.1.9 Any member of the toxicology section who performs examinations of alcoholic beverages will be required to complete Module 12 (Ethanol Content of Alcoholic Beverages).

1.1.10 The program culminates in the final competency exercise which includes a practical test, technical final, and a mock trial. For forensic toxicologists, the final competency also includes a pharmacology/toxicology oral examination.

1.2 Coordination of the Program

1.2.1 The TC is usually a supervisor in each laboratory however, this role may be delegated to a competent, experienced Forensic Scientist or Forensic Toxicologist.

1.2.2 The TC will be responsible for the overall training but may delegate certain duties and blocks of instruction to other individuals.

1.3 Training Period

1.3.1 The length of the training period is highly variable, and the length of training will be left to the determination of the TC and the PM. Some individual trainees may require less time than others depending on experience, education, and learning ability. The training period is usually completed within 12 months for forensic scientists and 18 months for forensic toxicologists.

1.4 Location of Training

1.4.1 Whenever practical, the bulk of an individual's training will occur in the laboratory to which they will be assigned.

1.5 Training Goals

The training should culminate such that the trainee has the following:

1.5.1 The knowledge of analytical chemistry.

1.5.2 The knowledge of the principles and practices of forensic toxicology related to the analysis of drugs and poisons within biological samples.

1.5.3 The knowledge of the theory and application of a variety of instruments used for the identification and quantitation of drugs.

1.5.4 The ability to perform accurate forensic toxicology analyses independently and proficiently.

1.5.5 The ability to skillfully present and defend analytical findings in courts of law.

1.6 Instructions to the Trainee

1.6.1 The trainee is expected to document their training activities and to provide a weekly progress report to the training coordinator. The progress report should also include upcoming training goals.

1.6.2 Throughout the training period, the trainee will observe data reviews and will perform data reviews of their own batches. The trainee may also conduct practice batch reviews on casework batches; however, these reviews are not documented on the casework batches (printed or electronic copies). This practice will expose the trainee to a wider variety of scenarios than they may be exposed to solely with training practice, exercises, and competency samples.

1.6.3 Once the trainee has demonstrated their competence to perform a particular analysis through the completion of specific training module(s), the trainee may be authorized by the PM on recommendation by the Section Supervisor, Group Supervisor, or TC to perform those analyses on casework. This authorization will be documented via MFR and the trainee's work authorization must be updated by the Laboratory Director prior to performing independent analyses on case work. For Evidence Receiving and Handling, the trainee must be added to the Delegation of Authority letter. Batch data run by trainees must be reviewed by a qualified examiner and this review will be documented on the batch summary worksheet. Trainees may not act as batch reviewers.

1.7 Instructions to Training Coordinators

1.7.1 The intent of the training manual is to define a program that will ensure every trainee receives a thorough foundation of certain basic principles and fundamentals necessary to the complete education of forensic lab specialists, forensic scientists, or toxicologists within the Toxicology

section. All of the listed topics, delineated in 1.1.8, must be incorporated into the training program for forensic scientists and toxicologists (refer to QM chapter on training for requirements).

1.7.2 It is recognized that some of the forensic laboratory specialists may only perform certain analyses. Therefore, they are only required to complete the modules (or portions thereof) associated with the type of work they perform, not necessarily all of the modules throughout the training program (refer to 1.1.8). See also QM 19.4.1.1.

1.7.3 The trainee is not permitted to perform supervised casework. "Supervised casework" is defined as the trainee performing active casework analysis under the direct supervision of a qualified examiner prior to the issuance of applicable work authorization(s).

1.7.4 Samples used for laboratory exercises may consist of blind controls, spiked samples, simulated case samples, or de-identified aliquots of appropriate samples retained for quality assurance purposes. Samples for competency exams shall, to the best of the TC's ability, consist of de-identified aliquots or spiked authentic matrix.

1.7.5 The TC is responsible for maintaining the Department's training program documentation during the training period. Each section of the training log must be dated and initialed upon the start and completion of the module. If any task is not completed, for any reason, this must be explained in the training file and approved by the PM.

1.7.5.1 The TC is authorized to substitute appropriate readings/information from alternate sources when required readings are not available. Appropriate sources include textbooks, peer-reviewed articles, informational videos, and websites. The PM shall be notified of any unavailable references or substitutions to implement changes into the Training Manual.

1.7.6 Once the trainee has satisfactorily completed the requirements of the program, the PM shall forward a written recommendation for certification to the Department Director.

1.7.7 If the trainee cannot meet the criteria expected of them during the training period, steps must be taken to determine and enact appropriate action.

1.7.8 The performance of the trainee will be evaluated throughout the course of the program. The evaluation will include the monthly training reports addressed in the QM. The TC must submit monthly evaluations through the Qualtrax workflow. Prior to submission of the workflow, the evaluation should be discussed between TC and trainee regarding previous accomplishments, deficiencies, and future objectives. The final report for each month will be maintained within Qualtrax.

1.8 Mock Trials

1.8.1 The training coordinator is responsible for ensuring that the trainee is thoroughly prepared for legal questioning. This can be done by a combination of mock trials, prearranged as well as impromptu question and answer sessions, and observation of courtroom testimony given by experienced examiners.

1.8.1.1 Refer to QM 19.7.2 for mock trials for Forensic Laboratory Specialists. Record formal mock trial practices in the training documentation.

1.8.2 The scheduling of practice mock trials is to be done by the TC. These are to be conducted throughout the training period.

1.9 Guidelines for Technical Examinations, Practical Test, and Final Mock Trial

1.9.1 Technical Examination

1.9.1.1 Prior to the final mock trial, a technical oral examination of the trainee will be conducted to ascertain the analytical knowledge of the individual. This will be limited to 3 hours.

1.9.1.2 After the examination, the evaluating members of the audience (minimally, the PM and TC) will discuss the trainee's performance.

1.9.1.3 The outcome of the examination will be "satisfactory" or "not satisfactory."

1.9.1.4 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to determine and enact appropriate action.

1.9.2 Pharmacology Technical Examination (Toxicologists only)

1.9.2.1 Prior to the final mock trial, a pharmacology technical oral examination of the toxicologist trainee will be conducted to ascertain their knowledge of pharmacology, toxicology, and interpretation of results. This will be limited to 3 hours.

1.9.2.2 After the examination, the evaluating members of the audience (minimally, the PM and TC) will discuss the trainee's performance.

1.9.2.3 The outcome of the examination will be "satisfactory" or "not satisfactory."

1.9.2.4 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to determine and enact appropriate action.

1.9.3 Practical Test

1.9.3.1 Following successful completion of all training modules, the trainee will be given a practical test to work as though it were a real case.

1.9.3.2 The practical test will be a typical case involving at least 3 analytical procedures (e.g., alcohol screen, immunoassay screen, and confirmation/quantitation).

1.9.3.3 Acceptable performance for alcohol analyses is ±0.004% w/v or ±6%, whichever is greater, of the expected value(s). Acceptable performance for drug analyses is ±20% of the expected value(s) (or within the published UoM for the individual drug(s)).

1.9.3.4 The trainee will generate an associated case file and Certificate of Analysis for the practical test.

1.9.4 Mock Trial

1.9.4.1 A video-recorded final mock trial will be conducted regarding the analysis (and interpretation for Forensic Toxicologists) of the practical test.

1.9.4.2 The PM must agree with the selection of all participants.

1.9.4.3 The atmosphere will be formal, that is, it will be conducted in the same manner as a real courtroom situation. This includes dress, conduct, protocol, and all other aspects. Answers and explanations are to be directed as to a lay jury or judge.

1.9.4.4 The mock trial will not exceed 2 hours.

1.9.4.5 Refer to the QM section on Mock Trials for guidelines and requirements.

1.10 Transition from Trainee to Examiner

1.10.1 After the new examiner has successfully completed this training, there follows a period of adjustment. The job of the TC is to ensure that this transition from trainee to qualified examiner takes place as smoothly as possible.

1.10.2 Casework will be introduced stepwise under the close supervision of a senior examiner.

1.10.3 The supervisor, TC, or designee will accompany and monitor the newly qualified examiner to court for the first few cases.

1.11 Continuing Education

1.11.1 All forensic lab specialists, forensic scientists, and toxicologists should participate in continuing education to maintain their skills and up-to-date knowledge in the field of forensic toxicology.

1.11.2 Examples of continuing education include, but are not limited to:

1.11.2.1 Attendance at meetings, workshops, or seminars.

1.11.2.2 Participation in study groups or scientific working groups.

1.11.2.3 Review of current literature.

1.11.2.4 Publication or presentation of research or case reports.

1.11.2.5 Education/training/teaching in the field of forensic toxicology.

1.11.2.6 Participation in specialized courses.

2 ORIENTATION

2.1 Minimum Requirements for Orientation

2.1.1 Assignment of a work area and TC.

2.1.1.1 Assist as needed with new employee access to:

2.1.1.1.1 DFS-provided computer & phone (Teams or similar, DFS group emails, etc.)

2.1.1.1.2 IT security training platform (e.g., KnowBe4)

2.1.1.1.3 State-required HR training (e.g., COV Learning Center)

2.1.1.1.4 Internal DFS document control (e.g., Qualtrax/Ideagen)

2.1.1.1.5 HR Payroll and Time Reporting (e.g., Cardinal)

2.1.2 Introduction to local operating facilities and personnel.

2.1.3 Coverage of the supervisor's Qualtrax onboarding checklist, to include:

2.1.3.1 Disaster Preparedness Training and Laboratory Tour

2.1.3.2 Safety Training

2.1.3.3 Drug Test

2.1.3.4 Buccal swab

2.1.3.5 Required Readings

2.1.3.5.1 Quality Manual

2.1.3.5.2 Administrative Policies

2.1.3.5.3 Regional Operating Procedures

2.1.3.5.4 Toxicology Procedures Manual (select sections assigned by TC, likely to include the Introduction, Toxicology Quality Guidelines, and Sampling Procedure chapters)

2.1.3.5.5 DFS Safety Manual

2.1.3.5.6 Organization of the Department of Forensic Science

2.1.4 Introduction to the technical capabilities of all DFS laboratories.

2.1.5 Explanation of the purpose of the training program including a review of the trainee's training plan and/or training timeline and what the trainee is expected to accomplish.

2.1.6 Explanation of the operations of local, state, and federal law enforcement agencies and court systems.

2.1.7 Clarification of the duties of forensic laboratory specialists, forensic scientists, and toxicologists within the Section.

2.1.8 Introduction to the LIMS system.

3 EVIDENCE RECEIVING AND HANDLING

3.1 Objectives

3.1.1 Understand physical evidence handling procedures used by DFS as detailed in the Quality Manual.

3.1.2 Understand physical evidence handling procedures pertinent to the Toxicology section.

3.1.3 Receive and process evidence for the Office of the Chief Medical Examiner (OCME), driving under the influence (DUI/DUID), and Toxicology-Other (police or TO) cases.

3.2 Methods of Instruction

3.2.1 Lectures and/or Self-Directed Study

3.2.1.1 Receiving and processing evidence

3.2.1.2 Evidence security

3.2.1.3 Chain of custody

3.2.1.4 LIMS

3.2.2 Required Reading

3.2.2.1 DFS Quality Manual (Service to the Customer and Evidence Handling chapters)

3.2.2.2 Toxicology Procedures Manual (Evidence Handling and Storage chapter)

3.2.2.3 Code of Virginia §§18.2-266, 18.2-268.1 – 18.2-268.7, 18.2-269

3.2.2.4 Additional Resources

3.2.2.4.1 ANSI/ASB BPR 156: *Best Practices for Specimen Collection and Preservation for Forensic Toxicology*

3.2.2.4.2 Virginia DFS Evidence Handling and Laboratory Capabilities Guide: Toxicology (Evidence Handling and Laboratory Capabilities Guide - Virginia Department of Forensic Science)

3.2.3 Demonstration

3.2.3.1 Evidence receiving and processing (to include sealing) will be observed from beginning to end and notes will be taken by the Trainee.

3.2.4 Initial Competency

3.2.4.1 The trainee will receive and process 5 simulated cases (a mixture of DUI/DUID, OCME, TO).

3.2.4.2 Successful completion of this task will be recorded on the "Toxicology Training Module Documentation Form" within the Evidence Handling Comment Grid with the TC's initials and date of completion (e.g., "Initial competency completed 5/21/2024").

3.2.4.3 Upon successful completion of the initial competency, the trainee will be approved in writing by the TC to complete the Laboratory Exercises with casework samples.

3.2.5 Laboratory Exercises

3.2.5.1 The trainee will maintain a list of processed samples for the training file.

3.2.5.2 The trainee will receive and process evidence for at least 20 OCME or TO samples and 20 DUI/DUID samples.

3.2.5.3 The trainee will seal at least 10 OCME or TO cases and at least 5 DUI/DUID cases.

3.3 Evaluation

3.3.1 Completion of written study questions.

3.3.2 Oral presentation followed by technical question/answer session.

3.4 Study Questions

3.4.1 List all procedural steps involving evidence receiving to final disposition for each of the following: DUI/DUID, OCME, TO cases.

3.4.2 Define the following terms: chain of custody, lock box, evidence seal, convenience packaging, RFLE, FS Lab#, LIMS.

3.4.3 Define a proper seal.

3.4.4 Who has access to the main evidence storage room? Toxicology storage refrigerators?

3.4.5 Who has access to your work area?

3.4.6 What actions are taken to ensure the proper preservation of evidence?

3.4.6.1 What are the acceptable temperatures for refrigerators? Freezers?

3.4.6.2 How often must the temperature be checked?

3.4.7 When is evidence returned to the originating agency?

3.4.8 List commonly encountered problems associated with the receipt of evidence and subsequent actions taken.

3.4.9 Specify the official chain-of-custody record for the following:

3.4.9.1 Submission of a DUI/DUID case with an RFLE if:

3.4.9.1.1 The case is a hand-to-hand submission.

3.4.9.1.2 The case was mailed in.

3.4.9.2 Submission of a DUI/DUID case without an RFLE.

3.4.9.3 Submission of an OCME case.

3.4.9.4 Placement of DUI/DUID samples into section storage.

3.4.9.5 Removal of an item from section storage for analysis.

3.4.9.6 Return of item to section storage after analysis.

3.4.10 List the prioritization of samples for an OCME case.

3.4.11 Describe what actions to take if you receive a low volume sample.

3.4.12 What should you do if you open a mailed-in DUI/DUID kit and discover that it contains hospital vials?

4 BASIC LABORATORY SKILLS

4.1 Objectives

4.1.1 Understand general chemistry principles required for preparation of laboratory solutions.

4.1.2 Understand proper use, maintenance, and quality assurance requirements for basic laboratory equipment (i.e., thermometers, heat blocks, evaporators, pipettes, balances, glassware).

4.1.3 Demonstrate proficiency for using basic laboratory equipment.

4.1.4 Understand preparation of sample homogenates.

4.1.5 Demonstrate proficiency for preparation of samples homogenates (where applicable, to include homogenization of clotted blood specimens and/or alternative matrices).

4.2 Methods of Instruction

4.2.1 Lectures and/or Self-Directed Study

4.2.1.1 Basic Laboratory Equipment Use and Quality Assurance.

4.2.1.2 Reagent Preparation.

4.2.1.3 Drug Standards Preparation.

4.2.1.4 Sample Homogenates and Dilutions.

4.2.2 Required Reading

4.2.2.1 DFS Quality Manual (Equipment chapter)

4.2.2.2 Toxicology Procedures Manual (Quality Assurance chapter to include all sections not covered in module-specific training (e.g., GC & QQQ will be covered in later modules))

4.2.3 Demonstration

4.2.3.1 Use of the pH meter, if applicable.

4.2.3.2 Pipette use, to include (where applicable) repeaters, positive displacement pipettes, and air displacement pipettes.

4.2.3.3 Required documentation for reagent and drug standards preparation and quality assurance logs for basic laboratory equipment. This includes electronic inventory or resource management tools (when available).

4.2.3.4 Reagent preparation.

4.2.3.5 Drug standards preparation (should include single component and multi-component preparations of calibrator, QC, or internal standard).

4.2.3.6 Sample homogenization and dilution.

4.2.4 Laboratory Exercises

4.2.4.1 Perform an intermediate (i.e. gravimetric) check on at least 3 calibrated pipettes of varying volume capacities (e.g., 10–100 µL, 100–1000 µL, 1–10 µL).

4.2.4.2 Perform a monthly balance check.

4.2.4.3 Calibrate the pH meter for use, if applicable.

4.2.4.4 Create mock documentation for the preparation of a specified reagent (e.g., buffer or mobile phase) of the TC or designee's choice. TC or designee will review the preparation

documentation and any applicable supporting documents for accuracy and completeness and document their review.

4.2.4.5 Prepare the appropriate documentation for a single or multi-component drug standard assigned by the TC.

4.2.4.6 Prepare appropriate documentation for a sample homogenate. If possible, perform a sample homogenization.

4.3 Evaluation

4.3.1 Completion of written study questions.

4.3.2 Laboratory Competency Testing

4.3.2.1 Prepare one to two reagents: one requiring a pH test and/or adjustment, at the discretion of the TC. The documentation must be accurate and the reagents must be considered acceptable for use upon verification by a qualified analyst (MUST be verified prior to use for reporting casework, not concurrently in a casework batch).

4.3.2.2 Prepare a calibrator, QC, and/or internal standard solution. The documentation must be accurate and the solution must be considered acceptable for use upon verification by a qualified analyst (MUST be verified prior to use for reporting casework, not concurrently in a casework batch).

4.3.3 Oral presentation followed by technical question/answer session.

4.4 Study Questions

4.4.1 What is NIST? Why is it important?

4.4.2 Solution Preparation

4.4.2.1 How much concentrated HCl is required to make 500 mL of 1 M HCl? Describe the preparation procedure in detail.

4.4.2.2 How much NaOH is required to make 1 L of a 1 M solution? Describe the preparation procedure in detail.

4.4.3 What is the difference between “to deliver” (TD) and “to contain” (TC)?

4.4.4 Describe the Toxicology quality assurance requirements and calibration/verification intervals for each of the following laboratory equipment:

4.4.4.1 Pipettes

4.4.4.2 Balances

4.4.4.3 Thermometers

4.4.4.4 Heat blocks

4.4.4.5 Evaporators

4.4.4.6 Refrigerators/Freezers

4.4.4.7 Diluters

4.4.5 Describe the preparation of sample homogenates to include clotted blood samples, gastric content, and a tissue.

4.4.6 Describe the preparation requirements for each of the following:

4.4.6.1 Reagent preparation

4.4.6.2 Calibrator preparation

4.4.6.3 QC preparation

4.4.6.4 Internal standard preparation

4.4.7 Describe how to perform the following dilutions of blood, urine, or other fluids (specify the fluid and appropriate diluent):

4.4.7.1 ½ dilution for alcohol analysis.

4.4.7.2 ¼ and 1/10 dilution for Opicoc analysis.

4.4.7.3 1/50 and 1/100 dilution for basic drug analysis.

4.4.7.4 What are the general recommendations for dilutions in the Toxicology section?

4.4.7.5 What are the conventions for notating dilutions? What is the difference between 1:4 dilution and ¼ dilution?

4.4.7.6 How are dilutions assigned?

4.4.7.7 How are dilutions documented?

4.4.7.8 How does one determine if a dilution is appropriate/allowed? (HINT: Where are the maximum allowable dilutions defined? Is there any flexibility for defined maximum dilutions?)

4.4.8 Explain the interconversion between mg/L, mg/dL, gm%, µg/mL, mg/mL, and ng/mL. Present 5 examples.

5 MEASUREMENT UNCERTAINTY

5.1 Objectives

5.1.1 To familiarize the trainee with traceability and its associated concepts.

5.1.2 To familiarize the trainee with concepts of measurement uncertainty.

5.2 Methods of Instruction

5.2.1 Lectures and/or Self-Directed Study

5.2.1.1 Presentations prepared by Chemistry Research Section Supervisor and DFS, available in Qualtrax.

5.2.1.1.1 Basic Statistics

5.2.1.1.2 Measurement Confidence

5.2.1.1.3 Toxicology Uncertainty of Measurement

5.2.2 Required Reading

5.2.2.1 Toxicology Procedures Manual (Estimation of the Measurement Uncertainty chapter)

5.2.2.2 Bell, S. *Measurement Uncertainty in Forensic Science – A Practical Guide*. CRC Press – Taylor and Francis Group.

5.2.2.2.1 Forensic Measurements, Metrology, and Uncertainty

5.2.2.2.2 Sources of Uncertainty

5.2.2.2.3 Foundational Concepts

5.2.2.2.4 Processes and Procedures

5.2.2.3 Additional Resources

5.2.2.3.1 Evaluation of Measurement Data – Guide to the Expression of Uncertainty in Measurement. BIPM. First Edition, 2008. (https://www.bipm.org/documents/20126/2071204/JCGM_100_2008_E.pdf/cb0ef43f-baa5-11cf-3f85-4dcd86f77bd6, https://www.bipm.org/en/committees/jc/jcgm/publications (accessed July 29, 2024)).

5.2.2.3.2 Bell, S. *A Beginner's Guide to Uncertainty of Measurement*. Measurement Good Practice Guide No. 11 (Issue 2).

5.2.2.3.3 ASCLD/LAB Policy on Measurement Uncertainty (AL-PD-3060)

5.2.2.3.4 ASCLD/LAB Policy on Measurement Traceability (AL-PD-3057)

5.2.2.3.5 ASCLD/LAB Guidance on Measurement Traceability (AL-PD-3058)

5.2.2.3.6 ASCLD/LAB Guidance on Estimation of Measurement Uncertainty – Overview (AL-PD-3061)

5.2.2.3.7 ASCLD/LAB Guidance on Estimation of Measurement Uncertainty – ANNEX A: Details on the NIST 8 Step Process (AL-PD-3062)

5.2.2.3.8 ASCLD/LAB Guidance on Measurement Traceability – Measurement Assurance (AL-PD-3059)

5.2.2.3.9 ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D: Toxicology Testing Discipline Example – Concentration of Ethanol in Antemortem Blood Specimen.

5.3 Evaluation

5.3.1 Completion of written study questions.

5.3.2 Oral presentation followed by technical question/answer session.

5.4 Study Questions

5.4.1 Define the following terms (use drawings/images if necessary):

5.4.1.1 ANAB

5.4.1.2 Mean

5.4.1.3 Median

5.4.1.4 Mode

5.4.1.5 Range

5.4.1.6 Accuracy

5.4.1.7 Precision

5.4.1.8 Gaussian Distribution

5.4.1.9 Confidence Interval

5.4.1.10 Coverage Factor

5.4.1.11 Measurement

5.4.1.12 Type A Evaluation

5.4.1.13 Type B Evaluation

5.4.2 Draw and explain a Gaussian distribution and how it relates to measurement uncertainty. Demonstrate two Gaussian distributions where one has high variability and one has low variability.

5.4.3 Obtain an uncertainty budget used in the Toxicology section. Define the elements and from where the information is obtained.

5.4.4 Within the Toxicology section, find a calibration standard that is traceable to NIST. Write a brief description of the traceability of that item.

5.4.5 Explain how to determine the coverage factor using Student's t-table. Provide the coverage factor for the following (may use Excel or a reference from the internet):

5.4.5.1 Alprazolam example: Confidence interval = 95.45% and Degrees of Freedom = 127

5.4.5.2 Blood alcohol example: Confidence interval = 99.73% and Degrees of Freedom = 1680

5.4.6 Using the coverage factors from the previous question, calculate the expanded uncertainty for alprazolam at 0.082 mg/L and blood alcohol at 0.128 %w/v. How would the reported values and the uncertainty be reported on the CoA?

6 ALCOHOL ANALYSIS BY HEADSPACE GAS CHROMATOGRAPHY

6.1 Objectives

6.1.1 Understand the theory, application, and practical aspects of gas chromatography (GC), to include headspace gas chromatography (HS-GC).

6.1.2 Comprehend the function and the specifics of operation of HS-GC.

6.1.3 Prepare specimens for analysis by HS-GC.

6.1.4 Operate the HS-GC.

6.1.5 Calibrate the instrument and quantitate ethanol, methanol, acetone, and isopropanol (2-propanol).

6.1.6 Interpret results by examining and explaining the chromatograms.

6.1.7 Understand the use of internal standards.

6.1.8 Demonstrate proficiency in analyzing blood alcohol cases.

6.1.9 Process and record results for OCME, DUI/DUID, and TO casework.

6.1.10 FLS (Optional)/FS & Tox (Required): Be proficient at batch preparation and review.

6.2 Methods of Instruction

6.2.1 Lectures and/or Self-Directed Study

6.2.1.1 Principles, Components, and Operation of HS-GC

6.2.1.2 Specimen preparation (dilution, internal standard)

6.2.1.3 Calibration and QC (Pre-run)

6.2.1.4 Result interpretation

6.2.1.5 FLS (Optional)/FS & Tox (Required): Parameters affecting the separation process and resolution of peaks using GC for analysis

6.2.1.6 FLS (Optional)/FS & Tox (Required): Types of injectors and injection techniques (applicable to GC)

6.2.1.7 FLS (Optional)/FS & Tox (Required): Types of columns (applicable to GC)

6.2.2 Required Reading

6.2.2.1 Toxicology Procedures Manual (Alcohols by Headspace Gas Chromatography chapter)

6.2.2.2 Garriott, J.C. et al. *Garriott's Medicolegal Aspects of Alcohol*. Lawyers and Judges Publishing Co., Inc.

6.2.2.2.1 Blood, Urine, and Other Fluid and Tissue Specimens for Alcohol Analyses

6.2.2.2.2 Analysis for Alcohol in Postmortem Specimens

6.2.2.2.3 Quality Assurance (Testing section)

6.2.2.2.4 Collection and Storage of Specimens for Alcohol Analysis

6.2.2.3 Levine, B. *Principles of Forensic Toxicology*. AACC Press.

6.2.2.3.1 Alcohol

6.2.2.3.2 Chromatography (Gas Chromatography sections)

6.2.2.4 Code of Virginia §18.2-266 – 18.2-269

6.2.2.5 Moffat, A.C. et al. *Clarke's Analysis of Drugs and Poisons*. The Pharmaceutical Press.

6.2.2.5.1 Alcohol, Drugs, and Driving
6.2.2.5.2 Gas Chromatography

6.2.2.6 Rood, D. *A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatography Systems*. Wiley-VCH.

6.2.2.7 Additional Resources

6.2.2.7.1 Skoog, D.A. et al. *Principles of Instrumental Analysis*. Brooks/Cole Thomson Learning.
6.2.2.7.1.1 An Introduction to Chromatographic Separations
6.2.2.7.1.2 Gas Chromatography

6.2.3 Demonstration

6.2.3.1 Blood alcohol analysis and operation of the HS-GC will be observed from beginning to end and notes will be taken by the Trainee.

6.2.3.2 Paperwork processing including batch assembly, control charting, and batch review.

6.2.4 Laboratory Exercises

6.2.4.1 Analyze at least one batch of 20 OCME biological specimens for ethanol. No less than five of the specimens will be positive for ethanol and at least one specimen will be negative.
6.2.4.1.1 The trainee will analyze the following (when feasible):

6.2.4.1.1.1 A minimum of one alternative matrix

6.2.4.1.1.1.1 Liver or gastric content or other tissue is preferred

6.2.4.1.1.1.2 Urine or vitreous are acceptable if tissues are not available

6.2.4.1.1.2 Sample dilution specimen (in the absence of an appropriate specimen for dilution or at the discretion of the TC, the trainee may provide a detailed description of how such dilutions are to be performed.)

6.2.4.2 Analyze at least one batch of 20 DUI/DUID blood specimens for ethanol. No less than ten of the specimens will be positive for ethanol and at least one specimen will be negative.
6.2.4.3 FLS (Optional)/FS & Tox (Required): Perform practice data review (do NOT mark up the data) of alcohol batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more BAC batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

6.3 Evaluation

6.3.1 Completion of written study questions.

6.3.2 Laboratory Competency Testing

6.3.2.1 The trainee will analyze at least 20 previously analyzed OCME biological specimens in the same manner as routine blood alcohol analysis. Trainee's results must fall within ±0.004% w/v or ±6%, whichever is greater, of the expected (reported or reanalysis) result.
6.3.2.2 The trainee will analyze at least 20 previously analyzed DUI/DUID blood specimens in the same manner as routine blood alcohol analysis. Trainee's results must fall within ±0.004% w/v or ±6%, whichever is greater, of the expected (reported or reanalysis) result.

6.3.2.3 FLS (Optional)/FS & Tox (Required): Pre-review (do NOT mark up the data) one BAC batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

6.3.3 Oral presentation followed by technical question/answer session.

6.4 Study Questions

6.4.1 Provide an overview of gas chromatography, including a schematic and explanation of the parts (including the injection system, column, and detector). Include an explanation of the principle and operation of HS-GC.

6.4.2 Describe the different types of GC stationary phases used in the Toxicology Section.

6.4.3 What is make-up gas? How and why is it used?

6.4.4 Explain the following statement: *response is proportional to the number of carbon atoms in the sample*. To what type(s) of detector is this statement applicable?

6.4.5 FLS (Optional)/FS & Tox (Required♦): What is column bleed?

6.4.6 FLS (Optional)/FS & Tox (Required♦): When and why are columns conditioned? Describe the process.

6.4.7 FLS (Optional)/FS & Tox (Required♦): Define the following terms:

6.4.7.1 Carrier gas

6.4.7.2 Height equivalent theoretical plate

6.4.7.3 Mobile phase

6.4.7.4 Resolution

6.4.7.5 Stationary phase

6.4.7.6 Partition coefficient

6.4.7.7 Retention time

6.4.7.8 Theoretical plates

6.4.7.9 Column efficiency

6.4.7.10 Van Deemter plot

6.4.7.11 Phase ratio

6.4.7.12 Selectivity

6.4.7.13 Flow rate

6.4.7.14 Relative retention time

6.4.7.15 Signal to noise ratio

6.4.8 FLS (Optional)/FS & Tox (Required♦): What are the possible causes and remedies for the following GC problems?

6.4.8.1 No peaks

6.4.8.2 Tailing peaks

6.4.8.3 Leading peaks

6.4.8.4 Split peaks

6.4.8.5 Baseline drift

6.4.9 Explain when calibration or recalibration of the HS-GC is necessary. How is recalibration accomplished?

6.4.10 What is the purpose of running a multicomponent alcohol control during the pre-run?

6.4.11 Manually calculate BAC based on the response of ethanol in a sample, internal standard, and calibrators.

6.4.12 What are the properties of a good internal standard?

6.4.13 Describe how chain of custody is maintained for samples in a batch. What is the appropriate documentation for the following actions?

6.4.13.1 Removal of an item from section storage for analysis.

6.4.13.2 Return of item to section storage after analysis.

6.4.13.3 You realize you need to homogenize a sample while you are in the process of aliquoting for your batch. You proceed to homogenize the sample prior to aliquoting it.

6.4.14 Explain what causes the blood alcohol concentration in a specimen to either decrease or increase. What measures can be taken to prevent this?

6.4.15 Describe the required components of a BAC batch.

6.4.16 FLS (Optional)/FS & Tox (Required): Discuss the relationship between the concentration of alcohol in blood with that in urine, serum, liver, and vitreous humor.

[♦*Required in either this module, or in the "Gas Chromatography" module.*]

7 IMMUNOASSAY

7.1 Objectives

7.1.1 Understand and explain immunoassay.

7.1.2 Understand the theory of commonly used immunoassay testing methods.

7.1.3 Understand the theory and practice of Immunalysis ELISA system.

7.1.4 Perform Immunalysis ELISA screening.

7.1.5 Interpret results by thoroughly explaining the calculations and instrument printouts.

7.1.6 Understand the quality control aspects of ELISA screening.

7.1.7 FLS (Optional)/FS & Tox (Required): Be proficient at batch preparation and review.

7.2 Methods of Instruction

7.2.1 Lectures and/or Self-Directed Study

7.2.1.1 Principles of immunoassay

7.2.1.2 Types of immunoassays

7.2.1.3 Components and operations of ELISA

7.2.1.4 Specimen preparation and analysis

7.2.1.5 Result interpretation

7.2.2 Required Reading

7.2.2.1 Toxicology Procedures Manual.

7.2.2.2 Levine, B. *Principles of Forensic Toxicology*. AACC Press.

7.2.2.2.1 Immunoassay

7.2.2.3 Moffat, A.C. *Clarke's Analysis of Drugs and Poisons*. The Pharmaceutical Press.

7.2.2.3.1 Immunoassays

7.2.2.4 Operator's Guide for the current ELISA system.

7.2.2.5 Crowther, J.R. The ELISA Guidebook. Humana Press.

7.2.3 Demonstration

7.2.3.1 ELISA analyses will be observed from beginning to end and notes will be taken by the Trainee.

7.2.4 Laboratory Exercises

7.2.4.1 Analyze a minimum of one batch of 10 biological specimens by ELISA screening for at least 10 different classes of drugs. At least 5 specimens will be above the cutoff concentration and at least one specimen below the cutoff.

7.2.4.2 FLS (Optional)/FS & Tox (Required): Perform practice data review (do NOT mark up the data) of ELISA batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more ELISA batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

NOTE: Toxicologist Trainees and their TCs will begin training on testing decisions for "review" cases at this time. Training on this aspect of the job should continue until TC and/or Toxicologist's

supervisor determines that proficiency in making testing decisions has been achieved. Successful completion of this training module serves as verification that this is complete.

7.3 Evaluation

7.3.1 Completion of written study questions.

7.3.2 Laboratory Competency Testing.

7.3.2.1 A series of at least 10 previously analyzed blood specimens will be presented to the Trainee for a routine DUID panel according to the Toxicology Procedures Manual. Qualitative results obtained by the Trainee must agree with previous results.

7.3.2.2 FLS (Optional)/FS & Tox (Required): Pre-review (do NOT mark up the data) one ELISA batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

7.3.3 Oral presentation followed by technical question/answer session.

7.4 Study Questions

7.4.1 Explain the advantages and disadvantages of screening for the presence of drugs.

7.4.2 Explain the following terms as they apply to ELISA

7.4.2.1 Antigen

7.4.2.2 Antibody

7.4.2.3 Monoclonal/polyclonal antibody

7.4.2.4 Microplate

7.4.2.5 Substrate

7.4.2.6 Horseradish peroxidase

7.4.2.7 Cross-reactivity

7.4.2.8 Cutoff concentration

7.4.2.9 Limit of detection

7.4.2.10 True-positive

7.4.2.11 False-positive

7.4.2.12 Sensitivity

7.4.2.13 False-negative

7.4.2.14 Specificity

7.4.3 Distinguish between homogeneous (e.g., enzyme multiplied immunoassay technique (EMIT)) and heterogeneous immunoassays (ELISA).

7.4.4 Explain cross-reactivity, stating advantages and disadvantages. Include the significance of immunoassay specificity for a specific drug vs. the specificity for a drug class.

7.4.5 Name the chemical compounds that is the primary target of the antibody in each of the DFS ELISA assays, and the respective cut-off level (PC) concentration.

7.4.6 For at least 5 of the immunoassay kits DFS uses, provide examples of drugs commonly encountered in casework that cross-react well, and those that do not cross-react well.

7.4.7 Explain the relationship between absorbance and the concentration of the drug being determined.

7.4.8 Explain the role of the negative control (NC), ½ cutoff (LPC), cutoff (PC), and high positive control (HPC).

7.4.9 Explain B/B_0. How is it calculated?

7.4.10 Explain the relationship between absorbance and the concentration of the drug being determined.

7.4.11 Describe the components of the ELISA kits and explain the purpose each.

7.4.12 Explain the effect(s) that the following situations could have on sample results:

7.4.12.1 Presence of bubble in aliquot taken by Tecan

7.4.12.2 Use of incorrect drug conjugate (e.g., use of methamphetamine drug conjugate from one kit lot with the wells from a different kit lot, use of opiate drug conjugate with THC wells)

7.4.12.3 Insufficient time allowed for competitive binding after addition of sample and drug conjugate

7.4.12.4 Incomplete plate washing

7.4.12.5 Plate incubation following TMB addition exceeds SOP-allowable time interval

7.4.13 Describe the required components of an ELISA batch.

8 CARBOXYHEMOGLOBIN SATURATION DETERMINATION

8.1 Objectives

8.1.1 Understand and explain the principles of ultraviolet-visible (UV/VIS) spectrophotometric measurements.

8.1.2 Understand the practice of UV/VIS spectrophotometry and the specifics of operation of the spectrophotometers at DFS. Understand the practice of carboxyhemoglobin confirmation with palladium chloride.

8.1.3 Perform instrumental analysis of carboxyhemoglobin using a UV/VIS spectrophotometer.

8.1.4 Interpret the results by thoroughly examining and explaining the instrument printout.

8.1.5 Understand the quality control aspects of spectrophotometric testing.

8.1.6 FLS (Optional)/FS & Tox (Required): Be proficient at batch preparation and review.

8.2 Methods of Instruction

8.2.1 Lectures and/or Self-Directed Study

8.2.1.1 Principles of spectrophotometry

8.2.1.2 Components and operation of the UV/VIS spectrophotometer

8.2.1.3 Specimen preparation and analysis

8.2.1.4 Results interpretation

8.2.1.5 Palladium chloride diffusion confirmation test

8.2.2 Required reading

8.2.2.1 Toxicology Procedures Manual.

8.2.2.2 Levine, B. *Principles of Forensic Toxicology*. AACC Press.

8.2.2.2.1 Spectrophotometry

8.2.2.3 Moffat, A.C. *Clarke's Analysis of Drugs and Poisons*. The Pharmaceutical Press.

8.2.2.3.1 Ultraviolet, Visible, and Fluorescence Spectrophotometry

8.2.2.4 Agilent "The Basics of UV-VIS Spectrophotometry – A Primer"

8.2.2.5 User Guide for current model of spectrophotometer in the lab (e.g., Agilent Cary 60 Spectrophotometer User's Guide)

8.2.2.6 Additional Resources

8.2.2.6.1 Skoog, D.A. et al. *Principles of Instrumental Analysis*. Brooks/Cole Thomson Learning.

8.2.2.6.1.1 An Introduction to Ultraviolet/Visible Molecular Absorption Spectrometry

8.2.2.6.1.2 Applications of Ultraviolet/Visible Molecular Absorption Spectrometry

8.2.2.6.2 Upstone, S.L. "Ultraviolet/Visible Light Absorption Spectrophotometry in Clinical Chemistry." *Encyclopedia of Analytical Chemistry*. John Wiley & Sons Ltd.

8.2.3 Demonstration

8.2.3.1 The use of the UV/VIS spectrophotometer for the semi-quantitative analysis of carboxyhemoglobin will be observed from beginning to end and notes will be taken by the trainee.

8.2.4 Laboratory Exercises

8.2.4.1 Analyze low, medium, and high controls for the presence of carboxyhemoglobin (COHb).

8.2.4.2 Screen a minimum of one batch of 5 blood specimens for the presence of COHb. At least 2 of the specimens will be positive and at least one specimen will be negative. In the absence of appropriate case samples, blind CO controls may be used for the laboratory exercises. Determine the approximate % saturation of each specimen.

8.2.4.3 Confirm the presence of CO using the palladium chloride diffusion test.

8.2.4.4 FLS (Optional)/FS & Tox (Required): Perform practice data review (do NOT mark up the data) of CO batches with at least two experienced examiners when possible. Pre-review (do NOT mark up the data) one or more CO batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

8.3 Evaluation

8.3.1.1 Completion of written study questions.

8.3.1.2 Laboratory Competency Testing.

8.3.1.2.1 A series of at least 5 previously analyzed blood specimens will be presented to the Trainee for COHb analysis. The results obtained by the Trainee must agree within 20% of the expected value. If previously analyzed casework specimens are not available, blind controls may be substituted at the TC's discretion.

8.3.1.2.2 FLS (Optional)/FS & Tox (Required): Pre-review (do NOT mark up the data) one CO batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

8.3.1.3 Oral presentation followed by a technical question/answer session.

8.4 Study Questions

8.4.1 Provide an overview of UV-Vis spectrophotometry. Include a schematic and explanation of the parts for an Agilent Cary 60 (or current UV-Vis instrument utilized by DFS), as well as an explanation of the principle and operation of the instrument.

8.4.2 What are the wavelength ranges for visible and ultraviolet electromagnetic radiation?

8.4.3 Explain what effects a change in solvent might have on the spectrum of a solute.

8.4.4 Discuss why a change in the pH of a solution can be important when using UV/VIS for analysis.

8.4.5 List and discuss some common sources of error in spectrophotometric measurements.

8.4.6 Define the following terms:

8.4.6.1 Wavelength

8.4.6.2 Absorbance

8.4.6.3 Transmittance

8.4.6.4 Excitation

8.4.6.5 Emission

8.4.6.6 Bandwidth

8.4.6.7 Beer-Lambert Law

8.4.7 In the semi-quantitative carboxyhemoglobin analysis, explain deoxyhemoglobin, oxyhemoglobin, methemoglobin, and carboxyhemoglobin.

8.4.8 How are the results reported for COHb on a certificate of analysis?

8.4.9 Explain the principle of the palladium chloride confirmation.

8.4.10 When should the analyst use lead acetate in the palladium chloride confirmation?

8.4.11 Describe a scenario in which a sample is determined to be unsuitable for analysis. Describe the required documentation associated with this determination.

8.4.12 What are the sample requirements for COHb analysis?

9 GAS CHROMATOGRAPHY (GC)

9.1 Objectives

9.1.1 FLS (Optional)/FS & Tox (Required): Understand the theory, practical aspects, and components of gas chromatography (GC), nitrogen phosphorus detectors (NPD), and mass spectrometry (MS).

9.1.2 FLS (Optional)/FS & Tox (Required): Become proficient in the use of GC and GC/MS for qualitative and quantitative analyses in Toxicology.

9.1.3 FLS (Optional)/FS & Tox (Required): Generate and evaluate chromatographic and mass spectral information to identify and quantitate drugs being analyzed.

9.1.4 FLS (Optional)/FS & Tox (Required): Understand and explain the criteria for acceptance of qualitative and quantitative data.

9.1.5 FLS (Optional)/FS & Tox (Required): Demonstrate a working knowledge of reporting qualitative and quantitative results in the manner used in the Toxicology Section.

9.1.6 FLS (Optional)/FS & Tox (Required): Be proficient at qualitative and quantitative batch preparation and review.

9.2 Methods of Instruction

9.2.1 Lectures and/or Self-Directed Study

9.2.1.1 GC theory, components, operation, and optimization

9.2.1.2 Types of detectors

9.2.1.3 Principles of mass spectrometry, ionization/source, detection

9.2.1.4 MS Components (sample inlets, ion sources, mass filters, detectors, vacuum systems)

9.2.1.5 Acquiring and evaluating mass spectra (spectral interpretation)

9.2.1.6 Operation of GC/MS in full scan and selective ion monitoring (SIM) modes

9.2.1.7 Use of libraries and databases

9.2.1.8 Use of GC and GC/MS software (e.g., ChemStation and MassHunter) to generate a calibration curve for quantitative data

9.2.2 Required Reading

9.2.2.1 Toxicology Procedures Manual.

9.2.2.2 Moffat, A.C. *Clarke's Analysis of Drugs and Poisons*. The Pharmaceutical Press.

9.2.2.2.1 Gas Chromatography

9.2.2.2.2 Mass Spectrometry

9.2.2.3 Levine, B. *Principles of Forensic Toxicology*. 2003. AACC Press.

9.2.2.3.1 Chromatography

9.2.2.3.2 Mass Spectrometry

9.2.2.4 Willard, H.H., et al. *Instrumental Methods of Analysis*. D. Van Nostrand Company, Inc.

9.2.2.4.1 Gas Chromatography

9.2.2.4.2 Mass Spectrometry

9.2.2.5 McLafferty, F. W. *Interpretation of Mass Spectra*. University Science Books.

9.2.2.5.1 Introduction (Chapter 1)

9.2.2.6 Additional Resources

9.2.2.6.1 ANSI/ASB Standard 113: *Standard for Identification Criteria in Forensic Toxicology*

9.2.2.6.2 ANSI/ASB Standard 098: *Standard for Mass Spectral Analysis in Forensic Toxicology*

9.2.2.6.3 Skoog, D.A. et al. *Principles of Instrumental Analysis*. Brooks/Cole Thomson Learning.

9.2.2.6.3.1 Gas Chromatography

9.2.2.6.3.2 Atomic Mass Spectrometry

9.2.2.6.3.3 Molecular Mass Spectrometry

9.2.2.6.4 Mills, T. and Robinson, JC. *Instrumental Data for Drug Analysis*. Vols. 1-7. Elsevier.

9.2.2.6.5 Hyver, K.J. et al. *High Resolution Gas Chromatography.* Hewlett-Packard Co.

9.2.2.6.6 Rood, D. *A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatography Systems*. Wiley-VCH.

9.2.2.6.7 Watson, J. T. *Introduction to Mass Spectrometry*. Lipincott-Raven.

9.2.2.6.8 Agilent GC/MS Instrument Manuals (Recommended models: 8890, 5975, & 5977)

9.2.3 Demonstration

9.2.3.1 The following techniques will be observed from beginning to end and notes will be taken by the Trainee: GC operation, qualitative drug identification by GC/NPD and GC/MS, and quantitative analysis by GC/NPD, GC/FID, and GC/MS SIM.

9.2.4 Laboratory Exercises

9.2.4.1 Perform a GC/MS autotune.

9.2.4.2 Determine the retention time and relative retention time (using the GC/NPD and methapyrilene as internal standard (where possible)) of basic drug mixes (may use data collected from previously analyzed casework data or trainee exercises).

9.2.4.3 Use GC/MS and mass spectral libraries to identify drugs and/or metabolites in 10 drug screens (10 total of base and acid/neutral). Review results with a qualified examiner to ensure all drugs and metabolites were correctly identified. Additional screens may be assigned by TC or designee as necessary.

9.2.4.4 Prepare a QC pack for a qualitative GC/MS screen using previously acquired data. Trainee should also provide a description of all the required documentation for an acceptable batch.

9.2.4.5 Perform practice data review (do NOT mark up the data) of both GC screen and GC quantitative batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more (each) GC screen and GC quantitative batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

9.2.4.6 Optional at the discretion of the TC: Perform routine maintenance of the GC/MS. May include but is not limited to: changing or adjusting the liner, septum, seals, gap column, transfer lines, and gold seal.

9.2.4.7 Optional at the discretion of the TC: Change the bead on an NPD. (*Due to the expensive nature of replacing the bead, as well as the lengthy conditioning process, the TC may instead have the trainee describe in detail how this is to be done, including conditioning the new bead, without requiring the trainee to physically perform this maintenance. However, the trainee should observe/participate in replacing a bead at the earliest opportunity.*)

9.3 Evaluation

9.3.1 Completion of written study questions.

9.3.2 Laboratory Competency Testing.

9.3.2.1.1 Trainee will perform qualitative data work-up for a batch of at least 5 previously analyzed biological specimens (GC/MS base or acidic/neutral screen, as specified by TC or designee). This shall include the preparation of the accompanying QC pack. Qualitative findings for specimens must agree with previously established results.

9.3.2.1.2 Trainee will perform quantitative data work-up for a batch of at least 5 previously analyzed biological specimens (GC/MS SIM or GC/NPD, as specified by TC or designee). This shall include the preparation of the accompanying QC pack. Quantitative findings for specimens must agree with previously established results.

9.3.2.1.3 Pre-review (do NOT mark up the data) one GC screen and one GC quantitative batch. Upon review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

9.3.2.1.4 Optional at the discretion of the TC: Clean a GC/MS source, including correct reassembly and installation of the source. Once the MS has been pumped down, the autotune must provide acceptable results for the competency test to be considered successful.

9.3.3 Oral presentation followed by technical question/answer session.

9.4 Study Questions

NOTE: Some study questions are covered in the optional section of the Blood Alcohol module. It is not intended for the trainee to replicate that work when already successfully performed. However, they must have covered the information in their oral presentation for the specific module during which they completed that element of the training, and they are responsible for covering the information during their technical evaluations (at least one mini-technical and the final technical). Alternatively, that portion of the training may be addressed during this module's training.

9.4.1 Draw a schematic diagram for a GC/MS and describe in detail the function of each component (including injector, columns commonly used in the Toxicology Section, and mass selective detector).

9.4.2 Draw a schematic diagram for a NPD and describe in detail the function of each component of the detector.

9.4.3 What are the advantages of using relative retention time rather than retention time for drug identification?

9.4.4 Describe the use of drug reference materials in the identification process.

9.4.5 Provide a brief overview of mass spectrometry.

9.4.6 Diagram and explain the functions of the components of a common EI source.

9.4.6.1 Are the ions formed positive or negative?

9.4.6.2 Do they have an even or odd number of electrons?

9.4.6.3 What is the ionization efficiency of this technique?

9.4.7 What vacuum conditions are necessary in the ionization source and the analyzing regions of a MS and why?

9.4.7.1 Describe how a rough pump works.

9.4.7.2 Describe how a turbomolecular pump works.

9.4.8 Describe the difference between full mass scan and selective ion monitoring in GC/MS analysis.

9.4.9 Explain the following MS terms:

9.4.9.1 Mass-to-charge ratio

9.4.9.2 Molecular ion

9.4.9.3 Precursor ion

9.4.9.4 Product ion

9.4.9.5 Base peak

9.4.9.6 Total ion chromatogram

9.4.9.7 Mass spectrum

9.4.9.8 Mass resolution

9.4.9.9 Relative abundance

9.4.9.10 Scan rate

9.4.9.11 Spectral tilting

9.4.10 Explain the Mathieu stability diagram.

9.4.11 Describe the importance of autotuning and explain the Autotune report for GC/MS.

9.4.12 What is an extracted ion profile? How would you use it in drug identification?

9.4.13 How does the probability-based-matching library search work?

9.4.14 Explain the following:

9.4.14.1 Threshold control (as applied to quantitative and qualitative analyses)

9.4.14.2 LOD

9.4.14.3 LOQ

9.4.14.4 ULOQ

9.4.15 Explain the SOP criteria concerning rejecting calibrator concentrations in a calibration curve.

9.4.16 Define and explain the following:

9.4.16.1 Blank and negative control

9.4.16.2 Positive control

9.4.16.3 Calibrator

9.4.17 How would the following be reported?

9.4.17.1 Drug concentration is greater than the ULOQ.

9.4.17.2 Drug concentration is below LOQ but above the threshold control and meets all qualitative acceptance criteria for reporting (e.g. retention time, ion ratios, chromatography).

9.4.17.3 Drug concentration is below LOQ and the threshold control and meets all qualitative acceptance criteria for reporting (e.g. retention time, ion ratios, chromatography).

9.4.17.4 Drug concentration is below the LOQ but above the threshold control and one ion ratio is unacceptable.

9.4.17.5 The LOQ changed (i.e. the lowest calibrator was removed). Drug concentration is below the new LOQ but above the threshold control and has acceptable ion ratios.

10 LIQUID CHROMATOGRAPHY (LC)

10.1 Objectives

10.1.1 FLS (Optional)/FS & Tox (Required): Understand the theory, practical aspects, and components of high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MSMS).

10.1.2 FLS (Optional)/FS & Tox (Required): Understand and explain the operation of the LCMSMS interface.

10.1.3 FLS (Optional)/FS & Tox (Required): Understand and explain ion formation.

10.1.4 FLS (Optional)/FS & Tox (Required): Understand and explain tandem mass spectrometry.

10.1.5 FLS (Optional)/FS & Tox (Required): Perform routine maintenance and tuning of the LCMSMS.

10.1.6 FLS (Optional)/FS & Tox (Required): Generate and evaluate mass spectral information to confirm and quantitate the drugs being analyzed.

10.1.7 FLS (Optional)/FS & Tox (Required): Become proficient in the use of LCMSMS for qualitative and quantitative analyses in Toxicology.

10.1.8 FLS (Optional)/FS & Tox (Required): Construct and apply calibration curves using LCMSMS software.

10.1.9 FLS (Optional)/FS & Tox (Required): Understand and explain the criteria for acceptance of qualitative and quantitative data.

10.1.10 FLS (Optional)/FS & Tox (Required): Demonstrate a working knowledge of reporting qualitative and quantitative results in the manner used in the Toxicology Section.

10.1.11 FLS (Optional)/FS & Tox (Required): Be proficient at batch preparation and review.

10.2 Methods of Instruction

10.2.1 Lectures and/or Self-Directed Study

10.2.1.1 Principles and components of HPLC

10.2.1.2 Types of LC columns

10.2.1.3 Optimization of liquid chromatography

10.2.1.4 Principles of tandem mass spectrometry

10.2.1.4.1 Ion Sources: ESI, APCI

10.2.1.4.2 Ion Focusing Optics/Lenses

10.2.1.4.3 QQQ: quadrupoles, collision cells

10.2.1.5 Modes of operation: MS1/MS2 Scan, SRM, MRM, Product Ion Scan, Precursor Ion Scan

10.2.1.6 MS components (sample inlets, focusing components, quadrupoles, collision cell, high energy dynode (HED))

10.2.1.7 Optimization of targets: purpose, parameters

10.2.1.8 Acquisition of data

10.2.1.9 LCMSMS Qualitative Analysis: overview, data interpretation, batch report generation

10.2.1.10 LCMSMS Quantitative Analysis: overview, data interpretation, batch report generation

10.2.2 Required Reading

10.2.2.1 Toxicology Procedures Manual.

10.2.2.2 Agilent Technologies 6400 Series QQQ LC/MS Techniques and Operation – Student Manual, pp. 12-28, 35-65, 93-112, 148-168, 300-320.

10.2.2.3 Agilent Technologies 6400 Series Triple Quad LC/MS System Manuals

10.2.2.3.1 Concepts Guide (Ch 2-3)

10.2.2.3.2 Maintenance Guide (pp 8-22, 67-72, 103-112)

10.2.2.3.3 Optimizer Technical Overview

10.2.2.4 Agilent LC-MSD Maintenance Videos

10.2.3 Levine, B. *Principles of Forensic Toxicology*. AACC Press.

10.2.3.1 Chromatography (High-Performance Liquid Chromatography section)

10.2.4 Moffat, A.C. *Clarke's Analysis of Drugs and Poisons*. The Pharmaceutical Press.

10.2.4.1 High Performance Liquid Chromatography

10.2.5 Additional Resources

10.2.5.1 Skoog, D.A. et al. *Principles of Instrumental Analysis*. Brooks/Cole Thomson Learning.

10.2.5.1.1 High-Performance Liquid Chromatography

10.2.5.1.2 Atomic Mass Spectrometry

10.2.5.1.3 Molecular Mass Spectrometry

10.2.6 Demonstration

10.2.6.1 LCMSMS operation and use of software for qualitative and quantitative reporting will be observed from beginning to end and notes will be taken by the Trainee.

10.2.7 Laboratory Exercises

10.2.7.1 Perform daily and weekly maintenance procedures. This is to include the evaluation of a Checktune, cleaning of the ion source, and the preparation of fresh solvents.

10.2.7.2 Review the results of Autotune and Checktune reports. Evaluate the reports.

10.2.7.3 Analyze data for either a fentanyl derivative or an NPS analysis (may use data collected from previous trainee exercises or from previously analyzed casework data). Evaluate acceptance based upon QC data for each drug in the mix.

10.2.7.4 Using Quantitative Analysis (MassHunter) software, generate QC pack data for a set of previously acquired data for quantitative and qualitative reporting. Trainee should also provide a description of all the required documentation for an acceptable batch.

10.2.7.5 Perform practice data review (do NOT mark up the data) of LCMSMS qualitative batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more LCMSMS qualitative batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

10.2.7.6 Perform practice data review (do NOT mark up the data) of LCMSMS quantitative batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more LCMSMS quantitative batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

10.2.7.7 Optional at the discretion of the TC: Use Lab Advisor to perform both leak and pressure tests of at least one LC system.

10.3 Evaluation

10.3.1 Completion of written study questions.

10.3.2 Laboratory Competency Testing.

10.3.2.1.1 Using Quantitative Analysis (MassHunter), generate and process a set of previously acquired data for quantitative and qualitative reporting. Results must align with the previously determined results.

10.3.2.1.2 Perform an LCMSMS qualitative on at least 5 training samples. Findings must agree with the expected qualitative results.

10.3.2.1.3 Pre-review (do NOT mark up the data) one LCMSMS qualitative batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

10.3.2.1.4 Pre-review (do NOT mark up the data) one LCMSMS quantitation batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

10.3.2.1.5 FLS (Optional)/FS & Tox (Required): When appropriate, change the column and put new mobile phase on an LCMSMS system. Perform a purge of the lines to remove any air bubbles. Run a check mix to demonstrate instrument is operating properly.

10.3.2.1.6 Optional at the discretion of the TC: Troubleshoot one or more leaks on an LCMSMS system. Follow with injection of a test mix (either extracted or non-extracted) to evaluate system performance.

10.3.3 Oral presentation followed by technical question/answer session.

10.4 Study Questions

10.4.1 Draw a schematic diagram for LCMSMS. Label and describe the function of each component.

10.4.2 Define the term “transition” as it relates to LCMSMS analysis and compare it to GC/MS SIM analysis. Explain how each provides appropriate specificity and quantitative information for the two types of analyses.

10.4.3 Describe factors that can affect peak resolution (e.g., particle size, column choice, mobile phase) in LCMSMS analysis. Using these factors, describe the steps you would take to resolve two co-eluting peaks.

10.4.4 Define the following:

10.4.4.1 Mobile phase

10.4.4.2 Capacity factor

10.4.4.3 Isocratic elution

10.4.4.4 Gradient elution

10.4.4.5 Normal phase HPLC

10.4.4.6 Ion chromatography

10.4.4.7 Reverse phase HPLC

10.4.4.8 Resolution

10.4.4.9 Transition ratio

10.4.5 Discuss the advantages and disadvantages of the following comparisons:

10.4.5.1 LC-MS vs. GC-EI-MS

10.4.5.2 LCMSMS SIM/scan vs LCMSMS SIM/SIM

10.4.6 Discuss the use of various buffers and acid additives within the mobile phase with respect to LCMSMS.

10.4.7 Diagram an electrospray ionization source (if not previously diagrammed for 10.4.1).

10.4.7.1 Explain the ionization process.

10.4.7.2 What is coulombic explosion?

10.4.7.3 What is the purpose of the drying gas?

10.4.8 Discuss ion suppression and enhancement and how they can affect LCMSMS analysis. Discuss indicators that ion suppression or enhancement are occurring within a batch of casework.

10.4.9 Explain the purpose of and how the Checktune and Autotune are performed for Agilent LCMSMS systems used in the Toxicology Section. When does the operator perform each of these activities?

10.4.10 Describe the acceptance criteria for a transition ratio. How is it calculated?

10.4.11 Describe the appropriate actions to take in the following scenarios:

10.4.11.1 Approximately 3 injections before your LCMSMS run finishes, the instrument shuts down due to insufficient mobile phase.

10.4.11.2 During Opicoc analysis, it is discovered that a case contained fentanyl greater than the ULOQ. The 5 cases run immediately following the high fentanyl case also contained reportable fentanyl.

10.4.11.3 You are working up your cannabinoids (SLE) data. One of the samples shows no peaks in any of the acquisition windows (no analyte, no IS).

10.4.11.4 You are working up your amphetamines data and notice that bupropion shifted over the course of the batch. Approximately one-quarter of the analyte peak was cut off for your last two QCs and several samples.

10.4.11.5 You are evaluating quantitative LCMSMS data. There appears to be carryover from the highest calibrator into the matrix blank injected immediately following.

10.4.12 How would the following be reported?

10.4.12.1 Drug concentration is below LOQ but above the threshold control. The transition ratio is not acceptable.

10.4.12.2 Drug concentration is below LOQ and the threshold control, and the transition ratio is not acceptable.

10.4.12.3 The LOQ changed (i.e. the lowest calibrator was removed). Drug concentration is below the new LOQ but above the threshold control and meets all qualitative acceptance criteria for reporting (e.g. retention time, ion ratios, chromatography).

10.4.12.4 During an Opicoc analysis, a case displays a peak for morphine above the LOQ, but the transition ratio is not acceptable and the peak displays poor chromatography. Additionally, both the morphine internal standard and the peak in the morphine window have shifted outside the acceptable range for retention time (both peaks shifted to the same degree in the same direction). 6AM is detected below the LOQ and above the threshold control and meets all reporting criteria.

10.4.12.5 Vitreous was extracted for Opicoc in the case described in 10.4.12.4. Morphine was not detected above the threshold control. 6AM is detected above the LOQ and meets all acceptance criteria.

10.4.12.6 One case sample in a recent fentanyl derivative batch contains a peak in the valeryl fentanyl window that meets all acceptance criteria. The threshold control for valeryl fentanyl was not acceptable for the batch.

11 EXTRACTIONS

11.1 Objectives

11.1.1 Understand the theoretical and practical aspects of extractions.

11.1.2 Extract representative compounds (basic, acidic, and neutral) from various matrices.

11.1.3 Understand the use of internal standards and quality controls as applied to qualitative and quantitative analyses.

11.1.4 Understand the role of derivatization.

11.1.5 Understand the requirements for screening and confirmation in forensic toxicology at DFS.

11.2 Methods of Instruction

11.2.1 Lectures and/or Self-Directed Study

11.2.1.1 Principles of extraction

11.2.1.2 Henderson-Hasselbach equation, acid-base equilibrium

11.2.1.3 Buffers and ionization

11.2.1.4 Extraction

11.2.1.5 Liquid-liquid extraction (LLE)

11.2.1.6 Solid phase extraction (SPE)

11.2.1.7 Supported liquid extraction (SLE)

11.2.1.8 Automated liquid handling system (ALH)

11.2.1.9 Derivatized extractions

11.2.1.10 Specimen preparation (dilution, internal standard, derivatization, hydrolysis)

11.2.2 Required Reading

11.2.2.1 Toxicology Procedures Manual.

11.2.2.2 Moffatt, A.C., editor. Clarke's Analysis of Drugs and Poisons. 3rd Ed. London: The Pharmaceutical Press. 2004 pp 80-108, 379-391, 425-499 (edition specific).

11.2.2.3 Levine, B. *Principles of Forensic Toxicology*. 2003. AACC Press.

11.2.2.3.1 Specimen Preparation

11.2.2.4 Juhascik, M. and Jenkins, A. Comparison of Liquid/Liquid and Solid Phase Extraction for Alkaline Drugs. Journal of Chromatographic Science. Vol. 47, August 2009, pp 553-557.

11.2.2.5 Hamilton MicroLab STAR User's Guide.

11.2.2.6 Solid Phase Extraction Techniques (United Chemical Technologies).

11.2.2.7 Biotage Isolute SLE+ User Guide

11.2.2.8 Additional References:

11.2.2.8.1 Mills, T. and Robinson, JC. Instrumental Data for Drug Analysis. 2nd Ed. Vols. 1-7, New York, Elsevier, 1987.

11.2.2.8.2 Knapp, D.R. *Handbook of Analytical Derivatization Reactions*. John Wiley & Sons.

11.2.2.8.3 Pierce Catalog. GC Derivatization and Labware. pp 497-526.

11.2.3 Demonstration

11.2.3.1 The following extraction techniques will be observed from beginning to end and notes will be taken by the Trainee, as applicable by laboratory location (i.e. if the trainee's assigned laboratory does not routinely perform any of the following, training will only be required prior to use): LLE, SPE, SLE, ALH, qualitative extraction, quantitative extraction, derivatization.

11.2.4 Laboratory Exercises

NOTE: If this module is utilized to train Forensic Laboratory Specialists to perform extractions without the expectation that they will be operating the instrument(s) independently for QA/QC purposes, the data work-up portion of the laboratory exercises and competency tests may not apply. A qualified analyst may perform the instrument operation and data analysis for the appropriate portions of the exercise.

11.2.4.1.1 Perform a LLE or SPE of acidic/neutral drug mixes and at least 3 previously analyzed biological specimens for qualitative analysis by GC/MS.

Perform quantitation of calibrators and controls for two or more commonly encountered basic drugs via GC-NPD or GC/MS.

11.2.4.1.2 Extract calibrators and controls for quantitative analysis by LCMSMS using each of the following extraction techniques: LLE, SPE, SLE.

11.2.4.1.3 Perform an extraction (e.g.,FentDeriv/Opicoc) of calibrators and controls using the ALH.

11.2.4.1.4 Perform a heavy metals screening analysis on appropriate controls.

11.2.4.1.5 Optional: Perform a volatile screening and confirmation of controls.

11.3 Evaluation

11.3.1 Completion of written study questions.

11.3.2 Laboratory Competency Testing.

NOTE: If this module is utilized to train Forensic Laboratory Specialists to perform extractions without the expectation that they will be operating the instrument(s) independently for casework, the data work-up portion of the laboratory exercises and competency tests may not apply. A qualified analyst may perform the instrument operation and data analysis for the appropriate portions of the test.

11.3.2.1 Perform a basic LLE or SPE (at the discretion of the TC or designee) of at least 5 previously analyzed biological specimens for analysis by GC/NPD (optional, at the discretion of the TC) and GC/MS. Qualitative findings must agree with previously established results.

11.3.2.2 Perform a LLE or SPE on a series of at least 5 previously analyzed biological specimens (base or acidic/neutral quantitation, as specified by TC or designee). Extracts will be run on the GC/MS and either the GC/NPD or GC/FID. Quantitative findings must agree within ±20% of the analyte's established target value (reported or reanalysis) or of the target analyte's measurement uncertainty for the current year.

11.3.2.3 Perform at least 2 LCMSMS quantitations of at least 5 biological specimens each, utilizing a variety of extraction techniques (e.g. LLE, SPE, SLE). The quantitations performed shall be specified by the TC or designee. Quantitative results must agree within ±20% of the analyte's

established target value (reported or reanalysis) or of the target analyte's measurement uncertainty for the current year.

11.3.2.4 Optional for labs that are not routinely utilizing GC methods for the listed compounds: Perform a GC quantitation of at least 5 biological specimens for carisoprodol, valproic acid, barbiturate, quetiapine, or hydroxyzine. Quantitative results must agree within ±20% of the analyte's established target value (reported or reanalysis) or of the target analyte's measurement uncertainty for the current year.

11.3.3 Oral presentation followed by technical question/answer session.

11.4 Study Questions

11.4.1 Describe LLE, SPE, and SLE. State the advantages and disadvantages of each extraction technique.

11.4.2 List and describe chemical forces which drive the movement of solute between aqueous and organic phases in LLE.

11.4.3 Explain the effects of pH on extractions.

11.4.4 List at least three different types of SPE sorbents and how they interact with the analytes being extracted.

11.4.5 List and explain the typical steps in an SPE procedure.

11.4.6 Explain the chemistry of SLE.

11.4.7 What are the quality assurance requirements for the automated liquid handling system? What are some things to consider when using the automated liquid handling system for analysis (e.g. Opicoc)?

11.4.8 Define the following terms

11.4.8.1 Matrix

11.4.8.2 Functional group

11.4.8.3 Polarity

11.4.8.4 Solvents

11.4.8.5 pH

11.4.8.6 pKa

11.4.8.7 Henderson-Hasselbach equation

11.4.8.8 Basic

11.4.8.9 Acidic

11.4.8.10 Neutral

11.4.8.11 Amphoteric molecules

11.4.8.12 Conjugate acid

11.4.8.13 Conjugate base

11.4.8.14 Internal standard

11.4.8.15 External standard

11.4.9 Describe silylation and methylation.

11.4.10 Describe and/or draw the derivative formed using the Toxicology Procedures Manual for a barbiturate (e.g., butalbital) and an analyte derivatized utilizing BSTFA + 1% TMCS.

11.4.11 Describe the appropriate actions to take in the following scenarios:

11.4.11.1 Opening the 2nd vial in a DUID case. Additionally, describe how to know if it is appropriate to open the 2nd vial.

11.4.11.2 A sample is consumed in analysis. Additionally, what are the volume thresholds for screening v. confirmation (e.g., for an extraction requiring 1 mL of sample, what do you do if there is only 0.9 mL of samples? Or only 0.4 mL of sample?)?

11.4.11.3 When pulling samples from administrative storage for the batch you are planning to extract, you notice one sample has limited volume. What, if anything, is/are appropriate action(s) to take?

11.4.11.4 You are performing the Amphetamines, Phentermine, and Designer Stimulants Quantitation and Confirmation by LCMSMS method on a batch of case samples. You transfer your 1-chlorobutane layer to clean, labeled tubes and transfer them into the TurboVap for evaporation. You realize that you forgot to add 0.2% HCl in IPA to the tubes.

11.4.11.5 You are performing the Benzodiazepines, Zolpidem, Zopiclone and Zaleplon Quantitation and Confirmation by LCMSMS method on a batch of case samples. After transferring your organic layer to clean, labeled tubes, you load your samples into the TurboVap for evaporation. When you initiate the nitrogen flow, you see a droplet of extract fly out of one of the tubes but you do not see where it lands.

12 ETHANOL CONTENT OF ALCOHOLIC BEVERAGES BY HEADSPACE GAS CHROMATOGRAPHY

12.1 Objectives

12.1.1 Demonstrate proficiency in the analysis of beverages for alcohol content.

12.2 Methods of Instruction

12.2.1 Lectures and/or Self-Directed Study

12.2.1.1 Chemical formulations and compositions of alcoholic beverages.

12.2.2 Required Reading

12.2.2.1 Toxicology Procedures Manual (Ethanol Content of Alcoholic Beverages by Headspace GC chapter)

12.2.2.2 Grossman, H.J. *Grossman's Guide to Wine, Beers, and Spirits*. Charles Scribner's Sons.

12.2.2.3 Lichine, A. *Alexis Lichne's Encyclopedia of Wines and Spirits*. Alfred A. Knopf.

12.2.2.4 Garriott, J.C. et al. *Garriott's Medicolegal Aspects of Alcohol*. Lawyers and Judges Publishing Co., Inc.

12.2.2.4.1 Chemistry of Alcoholic Beverages

12.2.2.5 Code of Virginia Title 4.1 Alcoholic Beverage Control Act, §4.1-100.

12.2.3 Demonstration

12.2.3.1 Alcoholic beverage analyses will be observed from beginning to end and notes will be taken by the Trainee.

12.2.4 Laboratory Exercises

12.2.4.1 Perform ethanol content analyses on 20 different alcoholic beverages.

12.2.4.2 FLS (Optional)/FS & Tox (Required): Perform practice data review (do NOT mark up the data) of ABC batches with at least two experienced examiners. Pre-review (do NOT mark up the data) one or more ABC batches, then solicit feedback from a qualified reviewer following their official review of the batch(es). (Trainee-developed batch review checklists may be utilized as desired.)

12.3 Evaluation

12.3.1 Completion of written study questions.

12.3.2 Laboratory Competency Testing.

12.3.2.1 A series of at least 20 different alcoholic beverages will be presented to the Trainee for a routine alcohol content determination. Quantitative results must agree within ±10 of the target value (reported or reanalysis).

12.3.2.2 FLS (Optional)/FS & Tox (Required): Pre-review (do NOT mark up the data) at least one ABC batch. Upon official review by a qualified analyst, no critical mistakes may be missed during the pre-review process.

12.3.3 Oral presentation followed by technical question/answer session.

12.4 Study Questions

12.4.1 Describe the differences between the Blood Alcohol method and the ABC Alcohol method.

12.4.2 Explain when calibration or recalibration of the HS-GC is necessary. How is recalibration accomplished? (NOTE: if answered during the BAC module, this question may be omitted.)

12.4.3 Describe the requirements that must be met in order to report a positive ethanol result.

12.4.4 Describe the ranges of alcohol content for the following alcoholic beverages: table wines, fortified wines, light beer, premium beer, malt liquors, special stouts, and distilled spirits.

12.4.5 Define the following:

12.4.5.1 Congeners

12.4.5.2 Proof

12.4.5.3 Fermentation

12.4.5.4 Mash

12.4.5.5 Distillation

12.4.6 What is the purpose of the Virginia Department of Alcoholic Beverage Control?

12.4.7 What are the common investigations for which ABC evidence is submitted?

12.4.8 Describe the accessioning process for ABC evidence.

13 DATA REVIEW AND CASE EXAMINATION

13.1 Objectives

13.1.1 To learn the process and documentation involved in data review, case examination, and technical review.

13.1.2 To learn the process for creating and releasing cases using LIMS.

13.2 Methods of Instruction

13.2.1 Data review and case examination training primarily learned by observing multiple certified examiners and performing training examinations that are critiqued by certified examiners.

13.2.2 Required Reading

13.2.2.1 Toxicology Procedures Manual (Toxicology Quality Guidelines chapter)

13.2.2.2 Quality Manual (Reporting Test Results and Monitoring Results chapter)

13.2.3 Demonstration

13.2.3.1 Trainee will observe and take notes of data (batch) review process with at least two experienced data reviewers. This may be considered complete if done with every training module, at the TC's discretion.

13.2.3.2 Trainee will observe and take notes of the case examination and review process with at least two experienced examiners for each case type (e.g., DUI/DUID, TO, OCME).

13.2.3.3 Trainee will observe and take notes on LIMS Certificate of Analysis (CoA) creation, technical review, and release with at least two experienced examiners.

13.2.4 Laboratory Exercises

13.2.4.1 Perform practice data review (do NOT mark up the data) on alcohol, immunoassay, drug screens, GC quantitation, GC/MS quantitation, and LCMSMS quantitation batches with at least two different examiners. (NOTE: These tasks are meant to be completed throughout the training program during the completion of each analytical module; if they are accomplished throughout training (and not skipped due to a modified training plan), this laboratory exercise may be considered successfully completed.)

13.2.4.2 Perform practice case examinations (do NOT mark up case data) on at least 10 non-implied consent cases with at least two different examiners (20 cases total minimum). Cases should be a variety to include (if available) homicide, drug overdose, drug-facilitated crime, and decomposition cases. No more than one medical examiner ethanol-only case may be included.

13.2.4.3 Perform at least 10 DUI/DUID case examinations with at least two different examiners (20 cases total minimum). No more than 5 ethanol only cases and 2 negative cases may be included.

13.2.4.4 The Trainee should document the review of at least 10 case files using the appropriate technical review form (TRF) or process. TRFs shall be marked as "Training" and retained in the Training File. Case files should be generated by multiple examiners, if possible, and include a variety of case types (e.g., DUI/DUID, TO, OCME). Any findings from the Trainee will be discussed with the TC before discussions with the case examiner. The case files shall be technically reviewed by a certified examiner prior to release.

13.2.4.5 Trainee should prepare UoM sheets and LIMS Certificates of Analysis (CoAs) for at least 5 cases from a variety of case types (e.g., OCME, TO, and DUID).

13.3 Evaluation

13.3.1 Completion of written study questions.

13.3.2 Laboratory Competency Testing

13.3.2.1 OCME/Toxicology-Other cases: The TC will select at least 20 cases that have not had final case examination performed. The Trainee will perform final case examination using a Toxicology Summary Worksheet (or other named document/process used for report generating purposes) marked as a "Training" case and submit cases for evaluation.

13.3.2.2 DUI/DUID cases: The TC will select at least 20 DUI/DUID cases that have not had final case examination performed. Trainee will perform final case examination using a Toxicology Summary Worksheet (or other named document/process used for report generating purposes) marked as a "Training" case and submit cases for evaluation.

13.3.2.3 The TC (or designee) will select a minimum of 10 cases that are ready for final technical review. The trainee will perform and document their review using the appropriate technical review form (TRF) or process. TRFs shall be marked as "Training" and retained in the Training File. Case files should include a variety of case types (e.g., DUI/DUID, TO, OCME) where possible. Any findings from the Trainee will be discussed with the TC before discussions with the case examiner. The case files shall be technically reviewed by a certified examiner prior to release, and the trainee may not have missed any critical mistakes.

13.4 Study Questions

13.4.1 What do the analyst date and time notations on the batch chain-of-custody indicate?

13.4.2 How many controls must be acceptable in a drug quantitation batch?

13.4.3 How is carryover monitored in a drug quantitation? What should be done when carryover is suspected?

13.4.4 Describe occasions when a drug may be reported as "present." "Present, greater than…"

13.4.5 An OCME case history states, "suspected overdose" and they requested an Abused panel. The blood morphine quantitation is 0.10 mg/L and 6AM is below the threshold level. As the case examiner, is this case complete? What other questions/analyses might you consider?

13.4.6 A methadone quantitation was performed on femoral blood and heart blood. Would you expect the methadone concentrations to be different and if so, why?

13.4.7 A sexual assault case has immunoassay blood benzodiazepine negative, urine benzodiazepine "pending" and urinary benzodiazepine confirmation "none detected." As final case examiner is benzodiazepine testing complete?

13.4.8 Hospital blood and urine are submitted in a DUI manslaughter case. The blood alcohol was 0.10% on two separate aliquots. Would you order a urine alcohol and why or why not?

13.4.9 You are examining a medical examiner case in which the subclavian blood ethanol was 0.287% w/v. Ethanol was none detected in the vitreous sample. Urine and liver specimens were also submitted. Is the testing complete?

13.4.10 You are examining a medical examiner case in which ethanol was 0.119% w/v. Opicoc analysis also found cocaine 0.27 mg/L and BE 1.9 mg/L. CE was not detected. As the case examiner, is this case complete? What other questions might you consider?

13.4.11 You are examining a DUID case. The ELISA DP was pending for methamphetamine and amphetamine was not detected. Confirmatory analysis resulted in 0.50 mg/L methamphetamine, 0.092 mg/L amphetamine, and 0.10 mg/L phentermine. As the case examiner, is this case complete? What other questions/analyses might you consider?

14 COURTROOM TESTIMONY

14.1 Objectives

14.1.1 To familiarize the trainee with the functions of a criminal courtroom proceeding.

14.1.2 To have the Trainee prepare a current curriculum vitae (or resume) and properly answer voir dire questioning.

14.1.3 To familiarize the Trainee with proper methods of presenting expert testimony.

14.2 Methods of Instruction

14.2.1 Required Reading

14.2.1.1 Saferstein, R. *Forensic Science Handbook*, Volume 1. Prentice Hall.

14.2.1.1.1 Legal Aspects of Forensic Science

14.2.1.2 Babitsky S. and J.J. Mangraviti. *How to Excel during Cross-Examination. Techniques for Experts that Work*. SEAK, Inc.

14.2.1.3 Kogan, J.D. "On Being a Good Expert Witness in a Criminal Case." *J For Sci*, Vol 23, No. 1, 1978, pp 190-200.

14.2.1.4 Kates, J.H. and H.L. Guttenplan. "Ethical Considerations in Forensic Science Services." *J For Sci*, Vol 28, No. 4, 1983, pp 972-976.

14.2.1.5 Keefe, J.F. "Forensic Sciences Services and the Criminal Justice System as Viewed by the Defense." *J For Sci,* Vol 24, No. 3, 1979, pp 673-680.

14.2.1.6 Lucas, D.M. "The Ethical Responsibilities of the Forensic Scientist: Exploring the Limits." *J For Sci*, Vol 34, No. 3, 1989, pp 719-729.

14.2.1.7 Saks, M.J. "Prevalence and Impact of Ethical Problems in Forensic Science." *J For Sci*, Vol 34, No. 3, 1989, pp 772-793.

14.2.1.8 Schroeder, O.C. "Ethical and Moral Dilemmas Confronting Forensic Scientists." *J For Sci*, Vol 29, No. 4, 1984, pp 966-986.

14.2.1.9 Wu, A. et al. "Minimal Standards for the Performance and Interpretation of Toxicology Tests in Legal Proceedings." *J For Sci*, Vol 44, No. 3, 1999, pp 516-522.

14.2.1.10 Saady, J. "Ethics for Toxicologists: An Examination of Conscience." *J Anal Tox*, Vol 25, 2001, pp 390-392.

14.2.1.11 Additional Resources

14.2.1.11.1 ANSI/ASB Best Practice Recommendation 037: *Guidelines for Opinions and Testimony in Forensic Toxicology*

14.2.2 Demonstration

14.2.2.1 Trainee will observe updating subpoena entries in LIMS and, where possible, the trainee should additionally observe entry of subpoenas in LIMS.

14.2.2.2 The trainee will observe expert courtroom testimonies. Discuss testimony with each examiner. Document each observed testimony with the name of the examiner, date, court, and notes reflecting the testimony and discussion. Where possible, the testimony observed should be applicable to the type of testimony the trainee is likely to provide once qualified.

14.2.2.3 Where possible, the trainee will observe pre-trial preparation with experienced examiners. Ideally, they would be able to observe several phone or in-person trial preparations.

Minimally, they should observe the process of pre-trial communication attempts with the attorney that issues the subpoena.

14.2.3 Practical Exercises

14.2.3.1 Complete curriculum vitae or resume.

14.2.3.2 Complete any DFS required qualification documents (e.g., Statement of Qualifications (SOQ)).

14.2.3.3 Mini mock trials (may be completed throughout other modules).

14.2.3.4 Optional: Trainee should review mock court testimony videos of past trainees. Ideally, they will watch these videos with either their TC or the person in the video. Discuss what was done well, and what could be done better in the future.

14.3 Evaluation

14.3.1 Completion of written study questions.

14.3.2 Courtroom exercises, to include one or more extensive voir dire sessions.

14.3.3 The trainee must be capable of answering questions on this module such as would be expected in a courtroom scenario. This may be completed through mini mock trials in other modules.

14.4 Study Questions

14.4.1 Discuss the role of the following during a trial:

14.4.1.1 Expert witness

14.4.1.2 Judge

14.4.1.3 Prosecutor

14.4.1.4 Defendant

14.4.1.5 Defense counsel

14.4.1.6 Jury

14.4.2 Define the following:

14.4.2.1 Voir dire

14.4.2.2 Direct examination

14.4.2.3 Cross examination

14.4.2.4 Redirect

14.4.2.5 Rebuttal witness

14.4.2.6 Chain of custody

14.4.2.7 Nolle prosequi (“nolle pros”)

14.4.3 Define the word “ethics.”

14.4.3.1 Why is it important in forensic science?

14.4.3.2 Investigate and describe the Code of Ethics for DFS, AAFS, ANAB, SOFT, and ABFT.

14.4.3.3 Give some examples of ethical violations and sanctions imposed by forensic organizations.

14.4.4 Prepare responses for the following questions (to be verbally answered during voir dire sessions with the TC or designee):

14.4.4.1 What is your name?

14.4.4.2 What is your occupation?

14.4.4.3 Who are you employed by?

14.4.4.4 How long have you been so employed?

14.4.4.5 What are your duties in this position?

14.4.4.6 What education and training do you possess that qualifies you to perform your duties?

14.4.4.7 What specific courses have you taken that are directly related to toxicology analysis?

14.4.4.8 How are these courses related? For example, what did you learn in your general chemistry course that aids you in the analysis of forensic toxicology samples?

14.4.4.9 What is the definition of an expert witness?

14.4.4.10 Is the university/college you graduated from accredited, and if so, by whom?

14.4.4.11 Who conducted your training?

14.4.4.12 What are his/her/their qualifications?

14.4.4.13 What literature do you read relating to your job?

14.4.4.14 How many analyses have you done on forensic cases?

14.4.4.15 Do you belong to a professional organization related to your occupation?

14.4.4.16 Have you written any articles or published materials dealing with your work?

14.4.5 If you are on the witness stand and an attorney objects to the question that has just been asked, what do you do? Who should you look at?

14.4.6 You are on the witness stand and the judge says, “You may go.” What do you do? Is there a question you should ask before leaving the witness stand? How should you ask that?

14.4.7 You are on the witness stand, you’ve answered a question, and the judge summarizes your statement incorrectly. (e.g., You have testified that your method is robust enough to report delta 9-THC, but you are able to detect and report the metabolite carboxy-delta 9-THC. The judge summarizes for the jury that your lab is not able to differentiate delta 9-THC from carboxy-delta 9-THC.) What do you do?

14.4.8 You finish testifying and have been excused. You return to the laboratory and are discussing court testimony with a colleague. You realize you made a mistake during your testimony. What do you do?

15 ALCOHOL PHARMACOLOGY, IMPAIRMENT, AND COURTROOM TESTIMONY

15.1 Objectives

15.1.1 To familiarize the Trainee with alcohol pharmacology (pharmacokinetics and pharmacodynamics) to include retrograde extrapolation and the use of the Widmark's equation.

15.1.2 To familiarize the trainee with testimony regarding ethanol effects and calculations.

15.1.3 Successful completion of a technical examination, a practical test, and a mock trial.

15.1.4 Attend the Robert F. Borkenstein Alcohol and Highway Safety Course provided by the Center for Forensic Science Research and Education. These are considered mandatory contingent on resources (funding, availability).

15.2 Required Reading

15.2.1 ANSI/ASB Best Practice Recommendation 122. Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology. First Edition, 2024.

15.2.2 Jones, A.W. (2011) Pharmacokinetics of Ethanol – Issues of Forensic Importance. Forensic Science Review, 23 (2) (July 2011), 92-132.

15.2.3 Jones, A.W. (2010) Evidence-based survey of the elimination rates of ethanol from blood with applications in forensic casework. Forensic Science International, 200, 1-20.

15.2.4 Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, ethanol specific chapter (edition dependent).

15.2.5 Garriott *Medicolegal Aspects of Alcohol*, Ch 2-4 (pharmacology), 13-15 (impairment), 17-19 (testimony) (edition dependent).

15.2.6 Dubowski, K.M. (1985) Absorption, Distribution, and Elimination of Alcohol: Highway Safety Aspects. Journal of Studies on Alcohol, Supplement No. 10.

15.2.7 Winek, C.L. et al (1996) Determination of absorption time of ethanol in social drinkers. Forensic Science International, 77, 196-177.

15.2.8 Jones, A.W. (1993) Disappearance Rate of Ethanol from the Blood of Human Subjects: Implications in Forensic Toxicology. Journal of Forensic Science, 38 (1), 104-118.

15.2.9 Jones, A.W. and Anderson, L. (1996) Influence of Age, Gender, and Blood-Alcohol Concentration on the Disappearance Rate of Alcohol from Blood in Drinking Drivers. Journal of Forensic Science, 41(6), 922-926.

15.2.10 Stowell, A.R. and Stowell, L.I. (1998) Estimation of blood alcohol concentrations after social drinking. Journal of Forensic Science, 43(1), 14-21.

15.2.11 Gullberg, R.G. and Predmore, D.B. (1982) Variation in blood alcohol concentration following the last drink. Journal of Police Science Administration, 10(3), 289-296.

15.2.12 Jones, A.W. and Jonsson, K.A. (1994) Food-induced lowering of blood-ethanol profiles and increased rate of elimination immediately after a meal. Journal of Forensic Science, 39(4), 1084-1093.

15.2.13 Jakus, J.T., Shajani, N.K., Image, B.A. (1992) Consumption of a large dose of alcohol in a short time span. Forensic Science International, 56(2), 113-125.

15.2.14 Jones, A.W., Jonsson, K.A., Neri, A. (1991) Peak blood-ethanol concentration and the time of its occurrence after rapid drinking on an empty stomach. Journal of Forensic Science, 36(2), 376-385.

15.2.15 Watkins, R.L. and Adler, E.V. (1993) The effect of food on alcohol absorption and elimination patterns. Journal of Forensic Science, 38(2), 285-291.

15.2.16 Moskowitz, H., Burns, M., Fiorentino, D., Smiley, A., Zador, P. (2000) Driver characteristics and impairment at various BACs. DOT Technical Document.

15.2.17 Moskowitz, H., Burns, M., Williams, A. (1985) Skills performance at low blood alcohol levels. Journal of Studies on Alcohol, 46(6), 482-485.

15.3 Study Questions

15.3.1 Describe zero and first order elimination. Diagram each.

15.3.2 Mr. Jones was in an accident at 0015 hrs. He admitted to drinking 3 beers rapidly at 2330hrs. He submitted to a breath test at 0200 hrs and the result was 0.20%w/v. What would his blood alcohol concentration have been at the time of the accident?

15.3.2.1 Further investigation revealed that he had his last drink at 2200hrs, but the accident still occurred at 0015hrs. Estimate his blood alcohol concentration at 0015hrs.

15.3.2.2 At trial, Mr. Jones claimed that after the accident, but before the officers arrived at the scene, he had consumed an unknown quantity of whiskey that he kept in his car. Estimate his blood alcohol concentration at 0015hrs.

15.3.3 How many beers would Mr. Jones (Height: 5’10”, Weight: 170lb, Age: 45, known alcoholic) have had to consume to reach 0.20%w/v? Assume two scenarios: 1) very rapid consumption (within 30 minutes) and 2) consumption over three hours.

15.3.4 Describe the effects of alcohol on human performance and how that correlates to driving skills.

15.3.5 Approximately how long would it take someone with a BAC of 0.31 g/210L to metabolize all the alcohol in the body?

15.3.6 Mrs. Brown (Age: 29, Height: 5’3”, Weight: 214lbs) was stopped at 2335hrs for weaving in her lane. Upon investigation, the officer charged her with DUI and conducted a breath alcohol analysis at 0017hrs with a result of 0.23g/210L. Mrs. Brown stated that she stopped drinking during happy hour which was at approximately 1800hrs and only consumed three glasses of wine.

15.3.6.1 How many standard drinks would Mrs. Brown had to have consumed at happy hour (assume all drinks consumed from 1700-1800 hours) to produce a result of 0.23g/210L approximately six hours later.

15.3.6.2 Is the scenario provided by the defendant consistent with the information provided by the officer and the blood alcohol measurement? Please explain.

15.4 Technical Examination

15.4.1 Prior to the mock trial, a technical oral examination of the trainee will be conducted to ascertain the alcohol pharmacological and impairment knowledge of the individual. This will be limited to 3 hours.

15.4.2 After the examination, the evaluating members of the audience (minimally, the Program Manager and TC) will discuss the trainee’s performance.

15.4.3 The outcome of the examination will be “satisfactory” or “not satisfactory”.

15.4.4 If the panel determines that the trainee’s performance was not satisfactory, steps must be taken to determine and enact an appropriate action.

15.5 Practical Test

15.5.1 Following successful completion of the technical examination, the Trainee will be given a practical test.

15.5.2 The practical test will be a scenario which will require a retrograde extrapolation and the use of Widmark's equation.

15.5.3 Acceptable performance is obtaining the expected result for the calculations.

15.6 Mock Trial

15.6.1 This mock trial may be combined with the final mock trial for forensic toxicologist trainees.

15.6.2 A recorded mock trial will be conducted using the scenario and calculation for the practical test.

15.6.3 The Toxicology Program Manager must agree with the selection of all participants.

15.6.4 The atmosphere will be formal, that is, it will be conducted in the same manner as a real courtroom situation. This includes dress, conduct, protocol and all other aspects. Answers and explanations are to be directed as to a lay jury or judge.

15.6.5 The mock trial will not exceed 2 hours.

15.6.6 The role of the prosecutor will be assumed by the training coordinator or designee.

15.6.7 The mock trial may be stopped at any time upon request of any of the involved parties.

15.6.8 After the court, supervision/management will assess the trainee's performance.

15.6.9 The outcome of the mock trial will be satisfactory or not satisfactory.

15.6.10 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

15.6.11 This evaluation will be immediately followed by a short performance critique.

15.6.12 The training coordinator will review the recording of the trial with the trainee as soon as possible. Other participants/observers should provide comments to the training coordinator as soon as possible.

16 PHARMACOLOGY AND TOXICOLOGY

16.1 Objectives

16.1.1 Display a working knowledge of the various categories of drugs encountered in toxicological analyses.

16.1.2 Understand the differences in interpretation for medical examiner (OCME) cases vs. driving under the influence of drugs (DUID) cases. Explain how the same drug concentration may be interpreted differently.

16.1.3 Know and understand the pharmacodynamic (PD) and pharmacokinetic (PK) properties of major drug classes.

16.1.4 Understand how the therapeutic, toxic, and lethal blood concentrations are assigned and used for populations, but may vary for an individual.

16.1.5 Explain the PD effects on human behavior and performance using blood drug concentrations as it pertains to court testimony and DUID cases.

16.1.6 Understand the process of postmortem redistribution, the interpretation of cases where this occurs, and which drugs are expected to undergo this process.

16.2 Methods of Instruction

16.2.1 Lectures and/or Self-Directed Study

16.2.1.1 SOFT Forensic Toxicology Review Course Lectures (2003)

16.2.1.2 Specific topics for each class of drugs.

16.2.1.2.1 General PK parameters (e.g., Vd, $t_{1/2}$, metabolism).

16.2.1.2.2 Major therapeutic and/or illicit uses.

16.2.1.2.3 Therapeutic effects.

16.2.1.2.4 Side effects.

16.2.1.2.5 Effects on driving.

16.2.1.2.6 Concentrations at which effects are observed.

16.2.1.2.7 Comparison of concentrations in DUID vs. postmortem cases.

16.2.1.2.8 Potential drug interactions.

16.2.1.2.9 Postmortem redistribution.

16.2.1.2.10 Practice trial testimony.

16.2.1.3 Literature review.

16.2.1.3.1 Levine, B. Principles of Forensic Toxicology. 2003.

16.2.1.3.2 Goodman and Gilman's The Pharmacological Bases of Therapeutics.

16.2.1.3.3 Garriott's Medicolegal Aspects of Alcohol.

16.2.1.3.4 SOFT Forensic Toxicology Review Course, Raleigh-Durham, NC. 2003.

16.2.1.3.5 National Highway Safety Traffic Administration. Drugs and Human Performance Fact Sheets. 2004.

16.2.1.3.6 The Effects of Drugs on Human Performance and Behavior. Forensic Science Review. 14. January 2002.

16.2.1.4 Discussion of interpretation and testimony.

16.2.1.5 Practice testimony on each drug class (mini mock trials).

16.2.1.6 Attend the Robert F. Borkenstein Effects of Drugs on Human Performance and Behavior Course provided by the Center for Forensic Science Research and Education. These are considered mandatory contingent on resources (funding, availability).

16.3 Evaluation

16.3.1 Written study questions on each class of drugs.

16.3.2 Mini mock trials on each class of drugs.

16.3.3 The Trainee must be capable of answering questions on each class of drugs such as would be expected in courtroom scenario.

16.4 Pharmacodynamics and Pharmacokinetics

16.4.1 Lectures and/or Self-Directed Study

16.4.1.1 Neurotransmission, drug-receptor interactions, and dose/response.

16.4.2 Required Reading

16.4.2.1 Levine, B. Principles of Forensic Toxiclogy. 2003. Ch. 4 (PK/PD, edition specific).

16.4.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 1-4, 12 (edition specific chapters; PK, PD, principles of therapeutics, principles of toxicology, neurotransmission).

16.4.3 Study Questions

16.4.3.1 Define pharmacokinetics and pharmacodynamics.

16.4.3.2 What factors influence absorption?

16.4.3.3 Will a weak base be absorbed primarily in the stomach or small intestine? Why? What about a weak acid?

16.4.3.4 Define bioavailability?

16.4.3.5 What is Vd? How is it calculated?

16.4.3.6 Describe zero and first order elimination. Diagram each (may use answer for Alcohol Pharmacology module).

16.4.3.7 Define first pass effect.

16.4.3.8 Give 5 examples of different routes of administration and a drug example for each. Describe how each route of administration would affect onset of action and peak blood concentration.

16.4.3.9 Give two examples of phase I and II reactions. Give a drug example for each.

16.4.3.10 Diagram a dose/response curve. What would be the effect of adding an antagonist? Add a non-competitive antagonist?

16.4.3.11 Diagram an neuronal synapse. Describe how reuptake inhibitors influence this environment.

16.4.3.12 Discuss the major structures of the brain that could be affected by drugs acting on the central nervous system.

16.4.3.13 What is therapeutic index? How is it calculated? Give an example of a drug with a high therapeutic index. Give an example of a drug with a low therapeutic index.

16.5 Opioids

16.5.1 Opioids to include, but not limited to, natural and synthetic opioids including fentanyl and fentanyl derivatives.

16.5.2 Required Reading

16.5.2.1 Levine, B. Principles of Forensic Toxicology. Ch 12 (edition specific, opioid chapter).

16.5.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch. 23-24 (edition specific, opioid related chapters).

16.5.2.3 NHTSA: Methadone, morphine.

16.5.3 Study Questions

16.5.3.1 Differentiate between the terms opiate, opioid, and narcotics.

16.5.3.2 Discuss the structure-activity relationship of morphine and its opiate analogs vs. the opiate antagonist, naloxone.

16.5.3.3 Which of the following are used to synthesize opioids? Give specific products.

16.5.3.3.1 Morphine.

16.5.3.3.2 Codeine.

16.5.3.3.3 Papaverine.

16.5.3.3.4 Noscopine.

16.5.3.3.5 Thebaine.

16.5.3.4 Discuss absorption, distribution, metabolism, and elimination (ADME) of heroin and fentanyl.

16.5.3.5 Discuss the role of codeine and 6AM in the determination of whether a death involved heroin.

16.5.3.6 What is the classical clinical presentation of acute opiate toxicity?

16.5.3.7 Discuss the pharmacological CNS effects of opiates that would be relevant in a DUID case.

16.6 Cocaine/Benzoylecgonine

16.6.1 Required Reading

16.6.1.1 Levine, B. Principles of Forensic Toxicology. Ch. 13 (edition specific, cocaine).

16.6.1.2 NHTSA: cocaine.

16.6.1.3 FSR: cocaine.

16.6.2 Study Questions

16.6.2.1 What is contraction band necrosis?

16.6.2.2 What are the effects of cocaine on catecholamines?

16.6.2.3 What is neurotransmitter depletion? How is it related to cocaine use?

16.6.2.4 What are the effects of cocaine on drivers at the following concentrations?

16.6.2.4.1 Cocaine 0.02 mg/L, benzoylecgonine 0.3 mg/L

16.6.2.4.2 Cocaine ND, benzoylecgonine 2.0 mg/L

16.7 Cannabinoids

16.7.1 Required Reading

16.7.1.1 Levine, B. Principles of Forensic Toxicology. Ch. 14 (edition specific, cannabinoids).

16.7.1.2 NHTSA: cannabinoids.

16.7.1.3 FSR: cannabinoids.

16.7.2 Study Questions

16.7.2.1 A Commonwealth Attorney calls to discuss the following cases. What would you say?

16.7.2.1.1 THC 0.001 mg/L, THC-COOH 0.02 mg/L. Driver pulled over for bad driving, officer witnessed suspect throw joint out of the window, performed poorly on SFSTs.

16.7.2.1.2 THC 0.001 mg/L, THC-COOH 0.02 mg/L. Driver pulled over for broken taillight, defendant admitted to smoking a joint the night before, performed well on SFSTs.

16.7.2.2 Is there an established relationship between THC blood concentration and driving impairment? Discuss why or why not.

16.7.2.3 What are the major metabolites of THC? Are the active/inactive? Which ones does DFS analyze and why?

16.7.2.4 Describe ADME of THC.

16.7.2.5 THC has a broad spectrum of pharmacological effects. Describe each. Can THC be classified in one drug category?

16.7.2.6 Describe the effects of THC on driving.

16.8 CNS Depressants

16.8.1 CNS depressants to include, but not limited to, benzodiazepines, barbiturates, carisoprodol, zolpidem, GHB.

16.8.2 Required Reading

16.8.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 11 (edition specific, CNS depressants).

16.8.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 17 (edition specific, CNS depressants).

16.8.2.3 NHTSA: Benzodiazepines, GHB.

16.8.2.4 FSR: Carisoprodol, GHB, zolpidem.

16.8.3 Study Questions

16.8.3.1 Make a table listing major CNS depressant drugs analyzed in DUID cases. Include the following information for each drug:

16.8.3.1.1 Dosage form.

16.8.3.1.2 Therapeutic uses.

16.8.3.1.3 Therapeutic, toxic, lethal ranges.

16.8.3.1.4 Half-life

16.8.3.1.5 Detection time in blood, urine.

16.8.3.1.6 Typical adverse side effects.

16.9 Sympathomimetic Amine

16.9.1 Sympathomimetic amines to include, but not limited to, methamphetamine, amphetamine, MDMA, ephedrine, and methylphenidate.

16.9.2 Required Reading

16.9.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 15 (edition specific, sympathomimetic amines).

16.9.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 10 (edition specific, sympathomimetic amines).

16.9.2.3 NHTSA: Methamphetamine.

16.9.2.4 FSR: methamphetamine, MDMA.

16.9.3 Study Questions

16.9.3.1 What are the common neurotransmitter involved in sympathomimetic pathways?

16.9.3.2 What are the common structural properties of these neurotransmitters?

16.9.3.3 How does hydroxylation affect their action?

16.9.3.4 Compare ADME for methamphetamine and MDMA. Include concentrations that contribute to observed effects and discuss tolerance.

16.9.3.5 What "rave" accessory is used to provide protection from a common MDMA side effect?

16.9.3.6 Discuss the noted effects of methylone (or other novel psychoactive substances like methylone for which DFS provides testing).

16.9.3.7 Discuss the effects of methamphetamine and MDMA on driving.

16.9.3.8 Sympathomimetic amines are usually present in racemic mixtures. Describe the different properties of d and l methamphetamine and MDMA.

16.10 **Hallucinogens**

16.10.1 Hallucinogens to include, but not limited to, LSD, PCP, ketamine, and psilocybin.

16.10.2 Required Reading

16.10.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 16 (edition specific, hallucinogens).

16.10.2.2 NHTSA: ketamine, LSD, PCP.

16.10.2.3 FSR: ketamine.

16.10.3 Study Questions

16.10.3.1 Which neurotransmitters are responsible for the hallucinogenic properties of compounds?

16.10.3.2 Compare ADME of LSD and PCP. Include dosage and detection times.

16.10.3.3 Discuss significant adverse effects of hallucinogenic drugs on driving.

16.10.3.4 What is the prevalence of hallucinogenic drug use in the general population?

16.11 Neuroleptics

16.11.1 Neuroleptics are also known as antipsychotics.

16.11.2 Required Reading

16.11.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 19 (edition specific, neuroleptics).

16.11.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 18 (edition specific, neuroleptics).

16.11.3 Study Questions

16.11.3.1 Give 2 examples each of old and new generation neuroleptics and describe ADME for each example.

16.11.3.2 What are some of the side effects of old and new generation neuroleptics?

16.11.3.3 What are some of the advantages of the new generation neuroleptics?

16.12 Antidepressants

16.12.1 Antidepressants to include, but not limited to, MAO, TCA, SSRI.

16.12.2 Required Reading

16.12.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 18 (edition specific, antidepressants).

16.12.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 19 (edition specific, antidepressants).

16.12.3 Study Questions

16.12.3.1 What are some of the side effects that would result from tricyclic antidepressant combined concentrations of 0.1 mg/L amitriptyline and 0.5 mg/L nortriptyline.

16.12.3.2 Compare and contrast mechanisms of actions, ADME, and side effects of TCAs, SSRIs, and MAOs.

16.13 Anticonvulsants

16.13.1 Anticonvulsants to include, but not limited to, phenytoin, carbamazepine, valproic acid, gabapentin, lamotrigine, and topiramate.

16.13.2 Required Reading

16.13.2.1 Levine, B. Principles of Forensic Toxicology. Ch. 17 (edition specific, anticonvulsants).

16.13.2.2 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 21 (edition specific, anticonvulsants).

16.13.3 Study Questions

16.13.3.1 Drugs used to control seizures have varied chemical structures. Describe the following: phenytoin, carbamazepine, valproic acid, gabapentin, lamotrigine, and topiramate.

16.13.3.2 Describe the neurological pathways of seizure control.

16.13.3.3 Describe lethal toxicities associated with seizure medications.

16.13.3.4 Describe the metabolism of carbamazepine and its significance.

16.13.3.5 Describe the adverse effects of seizure medication on driving.

16.13.3.6 In OCME cases, what is the most important reason for the analysis of seizure medication?

16.14 Antihistamines/NSAIDs

16.14.1 Antihistamines/NSAIDs to include, but not limited to, diphenhydramine, promethazine, dextromethorphan, acetaminophen, and acetylsalicylic acid.

16.14.2 Required Reading

16.14.2.1 Goodman and Gilman's The Pharmacological Basis of Therapeutics. Ch. 25, 27 (edition specific, antihistamines and NSAIDs).

16.14.2.2 NHTSA: diphenhydramine, dextromethorphan.

16.14.3 Study Questions

16.14.3.1 Make a table of histamine receptors including localization within the body, antagonists associated with each and therapeutic uses, therapeutic/toxic/lethal levels, therapeutic effects and effects on driving for each antagonist.

16.14.3.2 Why do antihistamines have anticholinergic effects?

16.14.3.3 Describe postmortem redistribution of antihistamines.

16.14.3.4 What antihistamines can be used in a DFC? What screening method is used to detect them? What is their detection time in blood and urine?