

Department of Forensic Science

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TOXICOLOGY TRAINING MANUAL
DEPARTMENT
OF
FORENSIC SCIENCE

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1 INTRODUCTION

1.1 Purpose and Scope

- 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.
- The manual is organized in modules and each module outlines the objectives, methods of instruction, modes of evaluation and study questions.
- 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.
- The training program provides exposure to court room testimony and legal aspects throughout the training and assists in developing the skills necessary to be an effective expert witness.
- The program evaluates the progress and performance of the trainee with each module. Each module includes laboratory exercises, competency tests and study questions. 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.
- The sequence in which the modules are presented should not necessarily be considered as a mandatory order of instruction.
- The trainee will complete a mini-technical final after the first 6 modules and a second mini-technical final on the remaining modules.
- It is recognized that some of the forensic laboratory specialists may only perform certain analyses. Therefore, FLS III and VI's are only required to complete the modules associated with the type of work they perform, not necessarily the entire training manual (e.g., FLS III who performs immunoassay screening must complete laboratory exercises, competency tests, study questions and oral presentation in the Immunoassay Module).
- Forensic scientists are expected to complete Modules 1-10 and 14-15.
- Forensic toxicologists are expected to complete Modules 1-10 and 12-15. Upon completion of module 13, the toxicologist's knowledge in pharmacology and toxicology will be assessed in a pharmacology technical oral examination.
- Any member of the toxicology section who performs examinations of alcoholic beverages will be required to complete Module 11 (Alcoholic Beverage Analysis). Since alcoholic beverage analyses are only conducted in the Central Laboratory by select personnel, most Trainees will not complete this section.
- The program culminates in the final competency exercise which includes a practical test, an analytical technical oral examination and a mock trial.

1.2 Coordination of the Program

- The training coordinator (TC) is usually a supervisor in each laboratory. A capable Forensic Scientist can also be delegated as the training coordinator, as necessary.
- The coordinator will be responsible for the overall training, but may delegate certain duties and blocks of instruction to other individuals.

1.3 Training Period

The length of the training period is a highly variable matter and will be left to the determination of the Toxicology Program Manager. Certain individuals may require less time than others, depending on experience, education or learning ability. However, the training period is usually completed within 12 months for forensic scientists and 18 months for forensic toxicologists.

1.4 Location of Training

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:

- The knowledge of analytical chemistry.
- The knowledge of the principles and practices of forensic toxicology related to the analysis of drugs and poisons within biological samples.
- The knowledge of the theory and application of a variety of instruments used for the identification and quantitation of drugs.
- The ability to perform accurate forensic toxicology analyses independently and proficiently.
- The ability to skillfully present and defend analytical findings in courts of law.

1.6 Instructions to the Trainee

- The trainee is expected to document all their training activity and to provide a weekly progress report to the training coordinator. The progress report should also include upcoming training goals.
- Once the trainee has demonstrated his/her competence to perform a particular analysis through the completion of specific training module(s), the trainee may be authorized by the Toxicology Program Manager on recommendation by the Section Supervisor to perform those analyses on case work. This authorization will be documented via MFR. Batch data run by trainees must be reviewed by a qualified examiner/FLS VI and this review be documented on the batch summary worksheet. Trainees may not act as batch reviewers.

1.7 Instructions to Training Coordinators

- As previously stated, the intent of the training manual is to define a program that will ensure each and every trainee receives certain basic principles and fundamentals necessary to the complete education of lab specialists, forensic scientists or toxicologists within the toxicology section. All of the listed topics must be incorporated into the program for forensic scientists and toxicologists (refer to QM 19.5.4).
- It is recognized that some of the forensic laboratory specialists may only perform certain analyses. Therefore, they are only required to complete the modules associated with the type of work they perform, not necessarily all the modules throughout the training program.
- Samples used for laboratory exercises may consist of blind controls, spiked samples, or simulated case samples. These options may be used at the TC's discretion.
- The training coordinator 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 specified task. If any task is not completed, for any reason, this must be explained in the training file and approved by the Toxicology Program Manager.

- Once the trainee has satisfactorily completed all of the requirements of the program, the Toxicology Program Manager shall forward a written recommendation for certification to the Department Director.
- If the trainee cannot meet the criteria expected of them during the training period, steps must be taken to effect appropriate action.
- The performance of the trainee will be evaluated during the course of the program. The evaluation may be in the form of the monthly training reports addressed in the QM (Appendix P). The TC must submit regular written evaluations of the trainee's progress to the Toxicology Program Manager. The coordinator is to discuss this evaluation with the trainee prior to forwarding it to the Toxicology Program Manager. Any relevant comments by either the trainee or coordinator are to be included with the report. The report should also be forwarded to the laboratory director.
 - The report should include both previous accomplishments and future objectives.
 - A copy of the report will be placed in the training file.

1.8 Mock Trials

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

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

- Technical Examination
 - 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.
 - After the examination, the evaluating members of the audience (minimally, the Program Manager and TC) will discuss the trainee's performance.
 - The outcome of the examination will be satisfactory or not satisfactory.
 - If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.
- Pharmacology Technical Examination (Toxicologists only)
 - 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.
 - After the examination, supervision/management will discuss the trainee's performance.
 - The outcome of the examination will be satisfactory or not satisfactory.
 - If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

- Practical Test
 - Following successful completion of all training modules, the trainee will be given a practical test to work as though it were a real case.
 - The practical test will be a typical case involving at least 3 analytical procedures (e.g., alcohol screen, immunoassay screen, and confirmation/quantitation).
 - Acceptable performance is $\pm 20\%$ of the expected values for drug analyses. Acceptable performance for alcohol analyses is $\pm 0.004\%$ w/v or 6%, whichever is greater, of the expected value.
 - The trainee will generate an associated case file and Certificate of Analysis for the practical test.
- Mock Trial
 - A recorded final mock trial will be conducted regarding the analysis of the practical test.
 - The Toxicology Program Manager must agree with the selection of all participants.
 - 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.
 - The mock trial will not exceed 2 hours.
 - The role of the prosecutor will be assumed by the training coordinator or designee.
 - The mock trial may be stopped at any time upon request of any of the involved parties.
 - After the court, supervision/management will assess the trainee's performance.
 - The outcome of the mock trial will be satisfactory or not satisfactory.
 - If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.
 - This evaluation will be immediately followed by a short performance critique.
 - 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.

1.10 Transition from Trainee to Examiner

- After the new examiner has successfully completed this training, there follows a period of adjustment. The job of the coordinator is to ensure that this transition from training to real life takes place as smoothly as possible.
- Casework will be introduced stepwise under the close supervision of a senior examiner.
- The supervisor, training coordinator or designee will accompany and monitor the newly qualified examiner to court for the first few cases.

1.11 Continuing Education

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

- Examples of continuing education include:
 - Attendance at meetings, workshops or seminars
 - Participation in study groups or scientific working groups
 - Review of current literature
 - Publication or presentation of research or case reports
 - Education/training/teaching in the field of forensic toxicology
 - Participation in specialized courses

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2 ORIENTATION

2.1 Minimum Requirements for Orientation

- Introduction to local operating facilities and personnel.
- Assignment of a work area.
- Coverage of the following:
 - Quality Manual
 - Administrative Policies
 - Regional Operating Procedures
 - Toxicology Procedures Manual
 - DFS Safety Manual
 - Organization of the Department of Forensic Science
- Introduction to the technical capabilities of all regional laboratories.
- Explanation of the purpose of the training program including an insight into the course of events and what the trainee is expected to accomplish.
- Explanation of the operation of local, state and federal law enforcement agencies and court systems.
- Clarification of the duties of forensic laboratory specialists, forensic scientists and toxicologists within the Section.
- Introduction to the LIMS system.

3 EVIDENCE RECEIVING AND HANDLING

3.1 Objectives

- Understand physical evidence handling procedures used by DFS as detailed in the Quality Manual.
- Understand physical evidence handling procedures pertinent to the toxicology section.
- Receive and process evidence for the Office of the Chief Medical Examiner (OCME), driving under the influence (DUI/DUID) and police cases.

3.2 Methods of Instruction

- Lectures
 - Receiving and processing evidence
 - Evidence security
 - Chain of custody
 - LIMS system
- Required Reading
 - Department of Forensic Science Quality Manual
 - Toxicology Procedures Manual
 - Code of Virginia, §18.2-266
 - Code of Virginia, §§18.2-268.1-18.2-268.3,18.2-268.5 - 18.2-268.7
 - Code of Virginia, §18.2-269
- Demonstration

Evidence receiving and processing will be observed from beginning to end and notes will be taken by the Trainee.
- Initial Competency
 - The Trainee will receive and process 5 simulated DUI/DUID samples and 5 OCME or Toxicology-Other samples.
 - 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 5/21/19”)
 - Upon completion of the initial competency, the trainee will be able to complete the Laboratory Exercises with casework samples.
- Laboratory Exercises
 - The Trainee will receive and process evidence for at least 20 ME or Toxicology-Other samples.

- The Trainee will receive and process at least 20 samples of DUI/DUID.
- The Trainee will seal at least 20 ME or Toxicology-Other samples.
- Maintain a list of processed samples for the training file.

3.3 Evaluation

- Completion of written study questions.
- Oral presentation followed by technical question/answer session.

3.4 Study Questions

- List all procedural steps involving evidence from receiving to final disposition for each of the following: DUI/DUID, ME and police cases.
- Define the following terms: chain of custody, lock box, evidence seal, convenience packaging, RFLE, FS lab #, LIMS.
- Define a proper seal.
- Who has access to the main evidence storage room? Toxicology storage refrigerators?
- Who has access to your work area?
- What actions are taken to ensure the proper preservation of evidence?
- When is evidence returned to the originating agency?
- List commonly encountered problems associated with receipt of evidence and subsequent actions taken.
- What is the official chain-of-custody record for the following:
 - Submission of a DUI/D case submitted with an RFLE
 - Submission of a DUI/D case submitted without an RFLE
 - Submission of an OCME case
 - Placement of DUI/D samples into section storage
 - Removal of item from section storage for analysis
 - Return of item to section storage after analysis

4 BLOOD ALCOHOL ANALYSIS

4.1 Objectives

- Understand the theory and application of headspace gas chromatography (GC).
- Comprehend the function and the specifics of operation of headspace GC.
- Prepare specimens for analysis by headspace GC.
- Operate the headspace GC.
- Calibrate the instrument and quantitate ethanol, methanol, acetone and 2-propanol.
- Interpret results by thoroughly examining and explaining the chromatograms.
- Understand the use of internal standards.
- Demonstrate proficiency by analyzing two runs (20 samples each) of blood alcohol cases.
- Process results and record results of medical examiner, DUI/DUID and police casework.
- Understand the uncertainty of measurement including how it is calculated and explained in court.

4.2 Methods of Instruction

- Lectures
 - Principles of headspace GC
 - Operation of the headspace GC
 - Specimen preparation (dilution, internal standard)
 - Calibration and QC (Pre-run)
 - Result interpretation
- Required Reading
 - Garriott, J. C., *Medicolegal Aspects of Alcohol*. 4th Ed. 2003, Lawyers & Judges Pub. Co, Inc. (Ch. 5, 6, 9.3.D)
 - Barry Levine (2003) *Principles of Forensic Toxicology*, Chapter 13. Alcohol (or the appropriately titled chapter)
 - Toxicology Procedures Manual
 - Code of Virginia (§18.2-266, -267, -268 (all subsections), -269)
 - Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 53-67.

- Demonstration
 - Blood alcohol analysis and operation of the headspace GC will be observed from beginning to end and notes will be taken by the Trainee.
 - Paperwork processing in medical examiner, DUI/DUID and police casework.
- Laboratory Exercises
 - Analyze one batch of 20 ME biological specimens for ethanol. At least 5 of the specimens will be positive for ethanol and at least one specimen will be negative.
 - Analyze one batch of 20 DUI blood specimens for ethanol. At least 10 of the specimens will be positive for ethanol and at least one specimen will be negative.

4.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing
 - A series of at least 20 previously analyzed ME biological specimens will be presented to the Trainee for a routine blood alcohol analysis. Trainee's results must fall within 0.004% w/v or 6%, whichever is greater, of the expected value.
 - A series of at least 20 previously analyzed DUI/DUID blood specimens will be presented to the Trainee for a routine blood alcohol analysis. Trainee's results must fall within 0.004% w/v or 6%, whichever is greater, of the expected value.
- Oral presentation followed by technical question/answer session

4.4 Study Questions

- Explain the principle and operation of headspace gas chromatography.
- Explain when calibration or recalibration of the headspace GC is necessary. How is recalibration accomplished?
- What is NIST? Why is it important?
- Discuss the relationship between the concentration of alcohol in blood with that in urine, serum, liver, and vitreous humor.
- Explain what causes the blood alcohol concentration in a specimen to either decrease or increase. What measures can be taken to prevent this?
- Explain the ethanol interconversion between mg/L, mg/dL, $\mu\text{g/mL}$ and gm%. Present 5 examples of each.
- What is the purpose of running a mixed volatile control during the prerun?
- Manually calculate BAC based on response of ethanol, internal standard and calibrators.
- What are the properties of a good internal standard?
- What is the UoM for the alcohol assay? How would you explain UoM in a courtroom?

5 IMMUNOASSAY

5.1 Objectives

- Understand and explain immunoassay.
- Understand the theory of commonly used immunoassay testing methods.
- Understand the theory and practice of Immunalysis ELISA system.
- Perform Immunalysis ELISA screening
- Interpret results by thoroughly explaining the calculations and instrument printouts
- Understand the quality control aspects of ELISA screening.

5.2 Methods of Instruction

- Lectures
 - Principles of immunoassay
 - Types of immunoassays
 - Components and operation of ELISA
 - Specimen preparation
 - Specimen analysis
 - Result interpretation
- Required Reading
 - Barry Levine (2003) *Principles of Forensic Toxicology*, pp 117-137.
 - Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 301-312.
 - Operator's Guide for the current ELISA system
 - Toxicology Procedures Manual

- Demonstration

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

- Laboratory Exercises

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

5.3 Evaluation

- Completion of written study questions.

- Laboratory Competency Testing

Qualitative – 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.

- Oral presentation followed by technical question/answer session

5.4 Study Questions

- Explain the advantages and disadvantages of screening for the presence of drugs.
- Explain the following terms as they apply to ELISA: antigen, antibody, monoclonal/polyclonal antibody, microplate, substrate, horseradish peroxidase, cross-reactivity, cutoff, limit of detection, true-positive, false-positive, sensitivity, false negative and specificity.
- Distinguish between homogeneous (e.g., enzyme multiplied immunoassay technique (EMIT)), and heterogeneous immunoassays (ELISA).
- Explain cross-reactivity stating advantages and disadvantages. Include the significance of immunoassay specificity for a specific drug vs. the specificity for a drug class.
- Name the chemical compound that is the primary target of the antibody in each of the ELISA assays, and the respective cut-off level (PC) concentration.
- Explain the relationship between absorbance and the concentration of the drug being determined.
- Explain B/B0. How is it calculated?
- Explain the role of the negative control, ½ cutoff, cutoff and positive control.
- Describe the components of the ELISA kits and explain the purpose of each.

6 SPECTROPHOTOMETRY

6.1 Objectives

- Understand and explain the principles of ultraviolet-visible (UV/VIS) spectrophotometric measurements.
- 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.
- Perform instrumental analysis of carboxyhemoglobin using a UV/VIS spectrophotometer.
- Interpret results by thoroughly examining and explaining the instrument printout.
- Understand the quality control aspects of spectrophotometric testing.

6.2 Methods of Instruction

- Lectures
 - Principles of spectrophotometry and spectrofluorometry
 - Components and operation of the UV/VIS spectrophotometer
 - Specimen preparation
 - Specimen analysis
 - Result interpretation
 - Palladium chloride diffusion confirmation test
- Required Reading
 - Barry Levine (2003) *Principles of Forensic Toxicology*, pp 79-88.
 - Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 313-327.
 - Toxicology Procedures Manual

- Demonstration

The use of UV/VIS spectrophotometry for the quantitative analyses of carbon monoxide will be observed from beginning to end and notes will be taken by the Trainee.

- Laboratory Exercises

- Analyze low, medium and high controls for the presence of carbon monoxide (CO).
- Screen one batch of 5 blood specimens for the presence of CO. 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. Calculate the % saturation of each specimen.
- Confirm the presence of CO using the palladium chloride diffusion test.

6.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing
 - A series of at least 5 previously analyzed blood specimens will be presented to the Trainee for CO analysis. The results obtained by the Trainee must agree within 20% of the reported value.
 - If there is a lack of previously analyzed case specimens, blind controls or supervised casework may be substituted at the TC's discretion.
- Oral presentation followed by technical question/answer session.

6.4 Study Questions

- What are the wavelength ranges for visible and ultraviolet electromagnetic radiation?
- Explain what effects a change in solvent might have on the spectrum of a solute.
- Discuss why a change in the pH of a solution can be important when using UV for analysis.
- List and discuss some common sources of error in spectrophotometric measurements.
- Define the following terms: wavelength, absorbance, transmittance, excitation, emission, bandwidth and Beer's law.
- In the quantitative carboxyhemoglobin analysis, explain deoxyhemoglobin, oxyhemoglobin, methemoglobin and carboxyhemoglobin.
- How are the results reported on the certificate of analysis for CO?
- Explain the principle of the palladium chloride confirmation.

7 QUALITATIVE DRUG SCREENS

7.1 Objectives

- Understand the theoretical and practical aspects of extractions.
- Understand the theory and practical aspects of gas chromatography (GC) and mass spectrometry (MS).
- Extract representative compounds (basic, acidic & neutral) from various matrices.
- Perform qualitative drug identification of biological specimen extractions using NPD gas chromatography.
- Perform qualitative drug identification of biological specimen extractions using full scan gas chromatography/mass spectrometry.
- Examine and interpret gas chromatographic printouts.
- Examine and interpret GC/MS results by explaining and comparing the mass spectra to libraries and databases.

7.2 Methods of Instruction

- Lecture
 - Principles of extraction
 - Henderson-Hasselbach equation, acid base equilibrium
 - Buffers and ionization
 - Extraction
 - Liquid-liquid extraction
 - Solid phase extraction (SPE)
 - Principles of gas chromatography
 - Components and operation of GC
 - Parameters affecting the separation process and resolution of peaks
 - Types of injectors and injection techniques
 - Types of columns
 - Types of detectors
 - GC optimization
 - Principles of mass spectrometry: ionization, source, detection
 - MS components (sample inlets, ion sources, mass filters, detectors, vacuum systems)
 - Acquiring and evaluating mass spectra

- Operation of GC/MS in full scan mode
- Use of libraries and databases
- Specimen preparation (dilution, internal standard)
- Required Reading
 - Solid Phase Extraction Techniques (United Chemical Technologies).
 - Toxicology Procedures Manual.
 - Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 80-108, pp 379-391, pp 425-499.
 - Willard, H.H., Merritt, L.L. Jr., Dean, J., Settle, F.A., *Instrumental Methods of Analysis*, 7th Ed. 1988, Wadsworth Pub Co., pp 540-578.
 - Barry Levine (2003) *Principles of Forensic Toxicology*, pp 67-78, 89-116, 139-153.
 - *Forensic Applications of Mass Spectrometry* in Saferstein's *Forensic Science Handbook*. Englewood Cliffs, NJ: Prentice Hall, 1982 pp 117-159.
 - Comparison of Liquid/Liquid and Solid Phase Extraction for Alkaline Drugs, Juhascik, M. and Jenkins, A., *Journal of Chromatographic Science*, Vol 47, August 2009, p. 553-557.
- References
 - Hyver, KJ. et al, *High Resolution Gas Chromatography*, 3rd Ed, 1989, Hewlett-Packard Co.
 - Rood, D. *A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatography Systems*, 3rd Revised Ed. 1999, Wiley-VCH.
 - Mills, T and Robinson JC. *Instrumental Data for Drug Analysis*, 2nd Edition. Volumes 1-7, New York: Elsevier, 1987.
 - Watson, J. T. *Introduction to Mass Spectrometry*. 3rd Ed. 1997. Lipincott-Raven.
 - *Handbook of Analytical Derivatization Reactions*, Daniel R. Knapp, John Wiley, New York, 1979.
 - Agilent Technologies GC/MS Instrument Manuals
- Demonstration

The following extraction techniques will be observed from beginning to end and notes will be taken by the Trainee: Liquid-liquid extraction, solid phase extraction and drug identification by GC/NPD and GC/MS.
- Laboratory exercises
 - Perform a liquid/liquid or SPE (dependent on technique performed at laboratory location) extraction of base screen drug mixes and 5 previously analyzed blood specimens for analysis by GC/NPD and GC/MS.
 - Perform a liquid/liquid extraction (or SPE) of acid/neutral mixes and 3 previously analyzed blood specimens for analysis by GC/MS.

- Determine the retention time and relative retention time (using the GC/NPD and methapyrilene as the internal standard) of basic drug mixes (use data collected from previous exercises or from previously analyzed casework data).
- Use GC/MS and mass spectral libraries to identify drugs and metabolites in 20 drug screens (20 total of base and acid/neutral). Review all cases with a qualified forensic scientist/toxicologist to ensure all drugs and metabolites were correctly identified.

7.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing

Liquid-liquid extraction or solid phase extraction (dependent upon technique performed at laboratory location) - a series of 5 previously analyzed blood specimens will be presented to the Trainee for base extraction, screens and confirmation by GC/NPD and GC/MS. Qualitative findings must agree with previously reported results.

- Oral presentation followed by technical question/answer session.

7.4 Study Questions

- Describe liquid-liquid and solid-phase extractions stating the advantages and disadvantages of each type.
- List and describe chemical forces which drive the movement of solute between aqueous and organic phases in liquid-liquid extraction.
- Explain the effects of pH on extractions.
- List at least three different types of SPE sorbents, and how they interact with the substances being extracted.
- List and explain the typical steps in an SPE procedure.
- Define the following terms: matrix; functional group; polarity; solvents; pH; pKa; Henderson-Hasselbach equation; basic, acidic, neutral and amphoteric molecules; conjugate acid, conjugate base; internal standard; external standard.
- Please provide a brief overview of gas chromatography.
- Draw a schematic diagram of a gas chromatograph and describe the function of each component. Provide detailed explanations of the injection system, column, and detectors commonly used in Toxicology.
- What are the advantages of using relative retention time for drug identification rather than retention time?
- Describe the different types of stationary phases used in the Toxicology Section.
- What is “make-up” gas? How and why is it used?
- Explain the following statement: *response is proportional to the number of carbon atoms in the sample*. What type(s) of detector is this statement applicable to?
- What are the possible causes and remedies for the following GC problems?
 - No peaks

- Tailing peaks
 - Leading peaks
 - Split peaks
 - Baseline drift
- What is column bleed?
 - When and why are columns conditioned? Describe the process.
 - Define the following terms:
 - Carrier gas
 - Height equivalent theoretical plate
 - Mobile phase
 - Resolution
 - Stationary phase
 - Partition coefficient
 - Retention time
 - Theoretical plates
 - Column efficiency
 - Make-up gas
 - Van Deemter plot
 - Phase ratio
 - Selectivity
 - Flow rate
 - Relative retention time
 - Signal to noise ratio
 - Describe the use of drug reference materials in the identification process.
 - Please provide a brief overview of mass spectrometry.
 - Draw a schematic diagram for a GC/MS and describe the function of each component.
 - Describe how a quadrupole mass filter operates.
 - Diagram and explain the functions of the components of a common EI source.
 - Are the ions formed positive or negative?
 - Do they have an even or odd number of electrons?
 - What is the ionization efficiency of this technique?
 - What vacuum conditions are necessary in the ionization source and the analyzing regions of a MS and why?
 - Describe how a rough pump works.
 - Describe how a turbomolecular pump works.
 - Describe the difference between full mass scans and selective ion monitoring.
 - Describe the importance of autotuning and explain the Autotune report.
 - Explain the following MS terms:
 - mass to charge ratio
 - molecular ion
 - parent ion

- base peak
 - total ion chromatogram
 - SIM
 - mass resolution
 - relative abundance
 - scan rate
 - spectral tilting
- What is an extracted ion profile? How would you use it in drug identification?
 - How does the probability-based-matching library search work?

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8 DRUG QUANTITATION**8.1 Objectives**

- Become proficient in the use of GC and GC/MS for quantitative analyses in Toxicology.
- Generate and evaluate chromatographic and mass spectral information to quantitate the drugs being analyzed.
- Perform routine maintenance on the gas chromatograph and mass spectrophotometer.
- Perform quantitative GC and GC/MS analyses of extracts from biological specimens for the presence of drugs.
- Generate accurate and precise quantitative results.
- Understand the use of internal standards and quality control as applied to GC and GC/MS.
- Perform derivatized drug quantitations. Understand the role of derivatization.
- Construct and apply calibration curves using GC and GC/MS software.
- Understand and explain the criteria for acceptance of quantitative GC and GC/MS data.
- Demonstrate a working knowledge of reporting quantitative GC and GC/MS results in the manner used in the toxicology section.

8.2 Methods of Instruction

- Lecture
 - Preparation of a calibration curve
 - Selected Ion Mode (SIM) of operation
 - Specimen preparation (dilution, internal standard, derivatization)
 - Spectral interpretation
 - Use of GC and GC/MS software (Chemstation and MassHunter) to generate a calibration curve for quantitative data
 - Derivatized extractions
- Required Reading
 - McLafferty, F. W., *Interpretation of Mass Spectra*, 3Ed. Chap 1.
 - Moffat, A. C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd Ed. The Pharmaceutical Press, London, 2004. pp 379-391.
 - Toxicology Procedures Manual
 - Barry Levine (2003) *Principles of Forensic Toxicology*, pp 89-116, 139-153.

- Demonstration
 - Extraction of a drug in a biological specimen with GC quantitation will be observed from beginning to end and notes will be taken by the Trainee.
 - Extraction and derivatization of a drug in biological specimens with GCMS SIM quantitation will be observed from beginning to end and notes will be taken by the Trainee.
- Laboratory Exercises
 - Perform an SPE opiate or cocaine quantitation of calibrators and controls.
 - Perform a GCMS SIM barbiturate quantitation (or other comparable GC/MS SIM quantitation) of calibrators and controls.
 - Spike, extract, and analyze a basic drug mix calibration curve and quality control samples in blood. Use the GC quantitative software to create a calibration curve and determine the quality control values.
 - Spike, extract, and analyze carisoprodol and meprobamate (or any acidic/neutral drug) calibration curve and quality control samples in blood. Use the GC quantitative software to create a calibration curve and determine the quality control values.
 - Perform daily routine maintenance of the GC/MS to include but not limited to changing or adjusting the autotune, liner, septum, seals, gap column, transfer lines, gold seal, etc.

8.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing
 - Perform two gas chromatography quantitations that are available in the Toxicology Procedures Manual. The quantitations used shall be chosen by the Training Coordinator. The quantitations should include 5 samples for analysis each.
 - Perform two GC/MS SIM quantitations that are available in the Toxicology Procedures Manual. The quantitations used shall be chosen by the Training Coordinator. The quantitations should include 5 samples for analysis each.
- Oral presentation followed by technical question/answer session.

8.4 Study Questions

- Explain LOD spike, LOD, LOQ, ULOQ as applied to GC and GC/MS quantitative measurements.
- Explain the SOP criteria concerning rejecting calibrator concentrations in a calibration curve.
- Define and explain the following:
 - Blank and negative control
 - Internal standard
 - External standard
 - Positive Control
 - Calibrator
- Describe silylation and methylation.

- Describe and/or draw the derivative formed using the Toxicology Procedures Manual for morphine, benzoylecgonine, and butalbital.
- How would the following be reported?
 - Drug concentration is greater than the ULOQ.
 - Drug concentration is below LOQ but above LOD spike and has acceptable ion ratios.
 - Drug concentration is below LOQ but above LOD spike and one ion ratio is unacceptable.
 - Drug concentration is below LOQ and LOD spike with one ion ratio unacceptable.

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9 LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY (LC-MS-MS)

9.1 Objectives

- Understand the theory, practical aspects, and components of high performance liquid chromatography instrumentation (HPLC) and tandem mass spectrometry (MS-MS).
- Understand and explain the operation of the LC-MS-MS interface.
- Understand and explain ion formation.
- Understand and explain tandem mass spectrometry.
- Perform routine maintenance and tuning of the LC-MS-MS.
- Generate and evaluate mass spectral information to confirm and quantitate the drugs being analyzed.
- Construct and apply calibration curves using LC-MS-MS software.
- Understand and explain the criteria for acceptance of quantitative LC-MS-MS data.
- Demonstrate a working knowledge of reporting quantitative LC-MS-MS results in the manner used in the toxicology section

9.2 Methods of Instruction

- Self-Directed Study
 - Principles and components of HPLC
 - Types of LC columns
 - Optimization of liquid chromatography
 - Principles of tandem mass spectrometry.
 - Ion Sources: ESI, APCI
 - Ion Focusing Optics/Lenses
 - QQQ: quadrupoles, collision cells
 - Modes of operation: MS1/MS2 Scan, SRM, MRM, Product Ion Scan, Precursor Ion Scan
 - MS components (sample inlets, focusing components, quadrupoles, collision cell, HED)
 - Optimization of targets: purpose, parameters
 - Acquisition of data
 - Qualitative results
 - Quantitative Analysis: overview, data interpretation, batch report generation
- Literature Reading
 - Virginia DFS Toxicology Procedures Manual

- Agilent Technologies 6400 Series QQQ LC/MS Techniques and Operation – Student Manual, pp. 12-28, 35-65, 93-112, 148-168, 300-320.
- Agilent Technologies 6400 Series Triple Quad LC/MS System Manuals
 - Concepts Guide – Ch. 2-3
 - Maintenance Guide – pp. 8-22, 67-72, 103-112
 - Optimizer Technical Overview
- Agilent Technologies LC-MSD Maintenance Videos

- Demonstration

Observe extraction of a drug from a biological specimen and analysis by LC-MS-MS. LC-MS-MS operation and use of quantitative software will be observed from beginning to end and notes will be taken by the Trainee.

- Laboratory Exercises

- 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.
- Review the results of Autotune and Checktune reports. Evaluate the reports.
- Using Quantitative Analysis, generate and process a set of previously acquired data.
- Spike, extract, and analyze an acetaminophen calibration curve and controls on a HPLC system, if this method is used in the training laboratory location.
- Spike, extract and analyze calibration curve and quality controls in blood using one of the LC-MS-MS methods from the Toxicology Procedures Manual. Use LC-MS-MS software to create a calibration curve and determine quality control values. Determine LOD spike, LOQ and ULOQ for each drug in the mix.

9.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing

Perform an LC-MS-MS quantitation (using an LC-MS-MS method from the Toxicology Procedures Manual) on at least 10 training samples. Acceptable performance is $\pm 20\%$ of the reported quantitation (or target value for spiked samples). Repeat using a different LC-MS-MS method.

- Oral presentation followed by technical question/answer session.

9.4 Study Questions

- Draw a schematic diagram of a HPLC system and describe the function of each component (include the photodiode array detector as the detection system).
- Define the following:
 - Mobile phase
 - Capacity factor
 - Isocratic elution

- Gradient elution
 - Normal phase HPLC
 - Ion chromatography
 - Reverse phase HPLC
 - Resolution
- Describe factors that can affect peak resolution (e.g., particle size, column choice, mobile phase). Using these factors, describe the steps you would take to resolve 2 co-eluting peaks.
 - Discuss the advantages and disadvantages of the following comparisons:
 - LC/MS vs. LC-MS-MS
 - GC-EI-MS vs. LC-MS-MS
 - Draw a schematic diagram for LC-MS-MS. Label and describe the functions of each component.
 - Discuss the use of various buffers and acid additives within the mobile phase with respect to LC-MS-MS.
 - Define the term transition and relate it to GC/MS SIM analysis. Explain how each provides appropriate specificity and quantitative information for the two types of analyses.
 - Diagram an electrospray ionization source (can use previously drawn schematic for LC-MS-MS).
 - Explain the ionization process.
 - What is coulombic explosion?
 - What is the purpose of the drying gas?
 - Discuss ion suppression and how it can affect LC-MS-MS analysis.
 - Explain the following with examples from the Agilent 6400 Series LC-MS-MS. When does the operator perform each of these activities?
 - Check-tune
 - Auto-tune

10 COURTROOM TESTIMONY

10.1 Objectives

- To familiarize the trainee with the functions of a criminal courtroom proceeding
- To have the trainee prepare a current curriculum vitae (or resume) and properly answer *voir dire* questioning
- To familiarize the trainee with proper methods of presenting expert testimony

10.2 Methods of Instruction

- Reading assignments
- Observation of expert testimony
- Answering study questions throughout training modules to lay jury or judge
- Practical exercises (mini-mock trials)
- Required Reading
 - Kuzmack, N.T., JD, MA. *Legal Aspects of Forensic Science* in Saferstein's *Forensic Science Handbook*. Englewood Cliffs, NJ: Prentice Hall, 1982 pp 1-27.
 - Babitsky S. and J. Mangraviti. *How to Excel during Cross-Examination. Techniques for Experts that Work*. Falmouth, MA: SEAK, 1997.
 - Kogan, J. *Being a Good Expert Witness in a Criminal Case*. *J For Sci* 23(1): 190-200, 1978.
 - Kates, James H. and Henry K. Guttenplan, Ph.D. *Ethical Considerations in Forensic Science Services* *J For Sci* 28(4): 972-976, 1983.
 - Keefe, J.F. *Forensic Sciences: Criminal Justice System Viewed by the Defense*. 12(2):59, 1980.
 - Lucas, Douglas M., M.Sc. *The Ethical Responsibilities of the Forensic Scientist: Exploring the Limits* *J For Sci* 34(3):719-729, 1989.
 - Saks, Michael J., Ph.D., M.S.L. *Prevalence and Impact of Ethical Problems in Forensic Science* *J For Sci* 34(3): 772-793, 1989.
 - Schroeder, Oliver C., J.D. *Ethical and Moral Dilemmas Confronting Forensic Scientists* *J For Sci* 29(4): 966-986, 1984.
 - Wu, A., Hill, D., Crouch, D., Hodnett, N., and H. McCurdy. *Minimal Standards for the Performance and Interpretation of Toxicology Tests in Legal Proceedings*. *J For Sci* 44(3): 516-522, 1999.
 - Saady, J. *Ethics for Toxicologists: An Examination of Conscience* *J Anal Tox* 25:390 - 392, 2001.
- Demonstration

The trainee will observe expert courtroom testimonies. Discuss testimony with each examiner. Document each observed testimony with name of examiner, date, court and notes reflecting the testimony and discussion.

- Practical Exercises
 - Complete required reading assignments
 - Complete curriculum vitae or resume
 - Mini mock trials

10.3 Evaluation

- Completion of written study questions.
- Courtroom Exercise

The Trainee must be capable of answering questions on this Module such as would be expected in a courtroom scenario.

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10.4 Study Questions

- Discuss the role of the following during a trial:
 - Expert witness
 - Judge
 - Prosecutor
 - Defendant
 - Defense counsel
 - Jury
- Define the following:
 - Voir dire
 - Direct examination
 - Cross examination
 - Redirect
 - Rebuttal witness
 - Chain of custody
- Define the word *ethics*.
 - Why is it important in forensic science?
 - Investigate and describe the Code of Ethics for DFS, AAFS, ASCLD/LAB, SOFT and ABFT.
 - Give some examples of ethical violations and sanctions imposed by forensic organizations.
- Verbally answer the following questions to the training coordinator or designee:
 - What is your name?
 - What is your occupation?
 - For whom do you work?
 - How long have you been so employed?
 - What are your duties in this occupation?
 - What education and training do you possess that qualifies you to perform your duties?
 - What specific courses have you taken that are directly related to toxicology analysis?
 - 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?
 - What is the definition of an expert witness?
 - Is the university/college you graduated from accredited, and if so, by whom?

- Who conducted your training?
- What are his/her/their qualifications?
- What literature do you read relating to your job?
- How many analyses have you done on forensic cases?
- Do you belong to a recognized society attesting to your qualifications in toxicology?
- Have you written any articles or published materials dealing with your work?

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11 ALCOHOLIC BEVERAGE ANALYSIS

11.1 Objectives

Demonstrate proficiency in the analysis of beverages for alcohol content.

11.2 Methods of Instruction

- Lectures

Chemical formulations and compositions of alcoholic beverages

- Literature Review

- Lembeck, H. *Grossman's Guide to Wine, Beers and Spirits*. New York: Charles Scribner's Sons, 1983.
- Lichine, A. *Alexis Lichine's Encyclopedia of Wines and Spirits*. New York: Alfred Knopf, Inc., 1983.
- Caplan, Y.H. and Goldberger, B.A., *Garriott's Medicolegal Aspects of Alcohol*. 6th Ed. 2015, Lawyers & Judges Publishing Co, Inc. (Chapter 1).
- Code of Virginia Title 4.1 Alcoholic Beverage Control Act, 4.1-100.

- Demonstration

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

- Laboratory Exercises

Perform ethanol content analyses on 20 different alcoholic beverages

11.3 Evaluation

- Completion of written study questions.
- Laboratory Competency Testing

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 previous results.

- Oral presentation followed by technical question/answer session.

11.4 Study Questions

- Explain when calibration or recalibration of the headspace GC is necessary. How is recalibration accomplished?
- What is NIST? Why is it important?
- 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.
- Define the following:
 - Congeners
 - Proof

- Fermentation
 - Mash
 - Distillation
-
- What is the purpose of the Virginia Department of Alcoholic Beverage Control?
 - What are common investigations in which ABC evidence is submitted?
 - Describe the accessioning process for ABC evidence.
 - Describe any differences between the Blood Alcohol method and the ABC Alcohol method.

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12 ALCOHOL PHARMACOLOGY, IMPAIRMENT, AND COURTROOM TESTIMONY**12.1 Objectives**

- To familiarize the trainee with alcohol pharmacology (pharmacokinetics and pharmacodynamics).
- To familiarize the trainee with retrograde extrapolation and the use of the Widmark's equation.
- To familiarize the trainee with testimony regarding ethanol effects and calculations.
- Successful completion of a technical examination, a practical test, and a mock trial.

12.2 Required Literature Reading

- Jones, A.W. (2011) Pharmacokinetics of Ethanol – Issues of Forensic Importance. *Forensic Science Review*, 23 (2) (July 2011), 92-132.
- 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.
- Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, ethanol specific chapter (edition dependent).
- *Garriott Medicolegal Aspects of Alcohol*, Ch 2-4 (pharmacology), 13-15 (impairment), 17-19 (testimony) (edition dependent).
- Dubowski, K.M. (1985) Absorption, Distribution, and Elimination of Alcohol: Highway Safety Aspects. *Journal of Studies on Alcohol*, Supplement No. 10.
- Winek, C.L. et al (1996) Determination of absorption time of ethanol in social drinkers. *Forensic Science International*, 77, 196-177.
- 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.
- 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.
- Stowell, A.R. and Stowell, L.I. (1998) Estimation of blood alcohol concentrations after social drinking. *Journal of Forensic Science*, 43(1), 14-21.
- 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.
- 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.
- 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.
- 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.
- 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.

- Moskowitz, H., Burns, M., Fiorentino, D., Smiley, A., Zador, P. (2000) Driver characteristics and impairment at various BACs. DOT Technical Document.
- Moskowitz, H., Burns, M., Williams, A. (1985) Skills performance at low blood alcohol levels. Journal of Studies on Alcohol, 46(6), 482-485.

12.3 Study Questions

- Describe zero and first order elimination. Diagram each.
- 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?
 - 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.
 - 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.
- 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.
- Describe the effects of alcohol on human performance and how that correlates to driving skills.
- Approximately how long would it take someone with a BAC of 0.31 g/210L to metabolize all the alcohol in the body?
- 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.
 - 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.
 - Is the scenario provided by the defendant consistent with the information provided by the officer and the blood alcohol measurement? Please explain.

12.4 Technical Examination

- 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.
- After the examination, the evaluating members of the audience (minimally, the Program Manager and TC) will discuss the trainee's performance.
- The outcome of the examination will be satisfactory or not satisfactory.
- If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

12.5 Practical Test

- Following successful completion of the technical examination, the trainee will be given a practical test.
- The practical test will be a scenario which will require a retrograde extrapolation and the use of Widmark's equation.
- Acceptable performance is obtaining the expected result for the extrapolation calculation.

12.6 Mock Trial

- This mock trial may be combined with the final mock trial for forensic toxicologist trainees.
- A recorded mock trial will be conducted using the scenario and calculation for the practical test.
- The Toxicology Program Manager must agree with the selection of all participants.
- 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.
- The mock trial will not exceed 2 hours.
- The role of the prosecutor will be assumed by the training coordinator or designee.
- The mock trial may be stopped at any time upon request of any of the involved parties.
- After the court, supervision/management will assess the trainee's performance.
- The outcome of the mock trial will be satisfactory or not satisfactory.
- If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.
- This evaluation will be immediately followed by a short performance critique.
- 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.

13 PHARMACOLOGY AND TOXICOLOGY

13.1 Objectives

- Display a working knowledge of the various categories of drugs encountered in toxicological analysis.
- Understand the differences in interpretation for medical examiner (ME) cases vs. driving under the influence of drug (DUID) cases. Explain how the same drug concentration may be interpreted differently.
- Know and understand the pharmacodynamic and pharmacokinetic properties of major drug classes.
- Understand how the therapeutic, toxic and lethal blood concentrations are assigned and used for populations, but may vary for an individual.
- Explain the pharmacodynamic effects on human behavior and performance using blood drug concentrations as it pertains to court testimony and DUID cases.
- Understand the process of postmortem redistribution, the interpretation of cases where this occurs, and which drugs are expected to undergo this process.

13.2 Methods of Instruction

- Lectures
 - SOFT Forensic Toxicology Review Course Lectures 2003
 - Specific lecture topics for each class of drugs
 - General pharmacokinetic parameters (V_d , $t_{1/2}$, metabolism)
 - Major therapeutic and/or illicit uses
 - Therapeutic effects
 - Side effects
 - Effects on driving
 - Concentrations at which effects are observed
 - Comparison of concentrations in DUID vs postmortem cases
 - Potential drug interactions
 - Postmortem redistribution
 - Practice trial testimony
- Literature Review
 - Barry Levine (2003) *Principles of Forensic Toxicology*.
 - Goodman and Gilman's *The Pharmacological Basis of Therapeutics*.
 - Garriott (2003) *Medicolegal Aspects of Alcohol*.

- SOFT Forensic Toxicology Review Course, Raleigh Durham, NC, 2003.
- *National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets*, 2004.
- *The Effects of Drugs on Human Performance and Behavior*, Forensic Science Review 14: Jan 2002.
- Discussion of interpretation and testimony.
- Practice testimony on each drug class (mini mock trials).
- Attend the Robert F. Borkestein Effects of Drugs on Human Performance and Behavior Course and the Postmortem Interpretive Toxicology Course provided by The Center for Forensic Science Research and Education. These are considered mandatory contingent on resources (funding, availability).

13.3 Evaluation

- Written study questions on each class or drugs.
- Mini mock trials on each class of drugs

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

13.4 Pharmacodynamics and Pharmacokinetics including Neurotransmission, Drug-Receptor Interactions, and Dose/Response

- Additional Lectures
 - SOFT Pharmacokinetics Workshop 2006
- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 4 (pharmacokinetic/pharmacodynamics)
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 1-4, 12 (pharmacokinetics, pharmacodynamics, principles of therapeutics, principles of toxicology, neurotransmission)
- Study Questions
 - Define pharmacokinetics.
 - Define pharmacodynamics.
 - What factors influence absorption?
 - Will a weak base be absorbed primarily in stomach or small intestine? Why? What about a weak acid?
 - Define bioavailability.
 - What is Vd? How is it calculated?
 - Describe zero and first order elimination. Diagram each.
 - Define first pass effect.

- 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.
- Give two examples of phase I and phase II reactions. Give a drug example for each.
- Diagram a dose/response curve. What would be the effect of adding an antagonist? Adding a non-competitive antagonist?
- Diagram a neuronal synapse. Describe how reuptake inhibitors influence this environment.
- Discuss the major structures of the brain that could be affected by drugs acting on the central nervous system.
- 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.

13.5 Opioids (Natural, Synthetic and Semisynthetic)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 12.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 23-24.
 - NHTSA: Methadone, morphine
- Study Questions
 - Differentiate between the terms opiate, opioid and narcotics.
 - Discuss the structure-activity relationship of morphine and its opiate analogs versus the opiate antagonist, naloxone.
 - Which of the following are used to synthesize opioids? Give specific products.
 - Morphine
 - Codeine
 - Papaverine
 - Noscopine
 - Thebaine
 - Discuss absorption, distribution, metabolism and elimination (ADME) of heroin.
 - Discuss the role of codeine and 6MAM in the determination of whether a death involved heroin.
 - What is the classical clinical presentation of acute opiate toxicity?
 - Discuss the pharmacologic CNS effects of opiates that would be relevant in a DUID case.

13.6 Cocaine/Benzoylcegonine

- Required Literature Reading

- Levine Principles of Forensic Toxicology, Ch 13.
- NHTSA: Cocaine
- FSR: Cocaine
- Study Questions
 - What is contraction band necrosis?
 - What are the effects of cocaine on catecholamines?
 - What is neurotransmitter depletion? How is it related to cocaine use?
 - What are the effects of cocaine on drivers at the following concentrations?
 - Cocaine 0.02 mg/L, benzoylecgonine 0.3 mg/L
 - Cocaine ND, benzoylecgonine 2.0 mg/L

13.7 Cannabinoids

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 14.
 - FSR: Cannabinoids
 - NHTSA: Cannabinoids
- Study Questions
 - A Commonwealth Attorney calls to discuss the following cases. What would you say?
 - THC 0.001 mg/L, THCA 0.02 mg/L. Driver pulled over for bad driving, officer witnessed suspect throw joint out of window, failed all FSTs.
 - THC 0.001 mg/L, THCA 0.02 mg/L. Driver pulled over for broken tail light, defendant admitted to smoking a joint the night before, performed fairly well FSTs.
 - Is there an established relationship between THC blood concentration and driving impairment? Discuss why or why not.
 - What are the major metabolites of THC? Are they active/inactive? Which one does DFS analyze and why?
 - Describe ADME of THC.
 - THC has a broad spectrum of pharmacologic effects. Describe each. Can THC be classified in one drug category?
 - Describe the effects of THC on driving.

13.8 CNS Depressants (Benzodiazepines, Barbiturates, Carisoprodol, Zolpidem, GHB, etc.)

- Required Literature Reading

- Levine Principles of Forensic Toxicology, Ch 11.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 17.
 - FSR: Benzodiazepines, GHB
 - NHTSA: Carisoprodol, GHB, zolpidem
- Study Questions
- Make a table listing major CNS depressant drugs analyzed in DUID cases. Include:
- Dosage form
 - Therapeutic uses
 - Therapeutic range
 - Toxic concentrations
 - Lethal concentrations
 - Half-life
 - Detection time in blood
 - Detection time in urine
 - Typical adverse side effects

13.9 Sympathomimetic Amines (Methamphetamine, Amphetamine, MDMA, Ephedrine, Methylphenidate)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 15.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 10.
 - FSR: Methamphetamine
 - NHTSA: Methamphetamine, MDMA
- Study Questions
 - What are the common neurotransmitters involved in sympathomimetic pathways?
 - What are the common structural properties of these neurotransmitters?
 - How does hydroxylation affect their action?
 - Compare ADME for methamphetamine and MDMA. Include concentrations that contribute to observed effects and discuss tolerance.
 - What "rave" accessory is used to provide protection from a common MDMA side effect and why is it used?
 - Discuss the noted effects of methylone (or other novel psychoactive substances like methylone for which DFS provides testing).
 - Discuss the effects of methamphetamine and MDMA on driving.
 - Sympathomimetic amines are usually present in racemic mixtures. Describe the different properties of d and l methamphetamine and MDMA.

13.10 Hallucinogens (LSD, PCP, Ketamine, Psilocybin)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 16.
 - FSR: Ketamine
 - NHTSA: Ketamine, LSD, PCP
- Study Questions
 - Which neurotransmitters are responsible for the hallucinogenic properties of compounds?
 - Compare ADME of LSD and PCP. Include dosage and detection times.
 - Discuss significant adverse effects of hallucinogenic drugs on driving.
 - What are the lethal toxic effects of hallucinogenic drugs?
 - What is the prevalence of hallucinogenic drug use in the general population?

13.11 Neuroleptics (Antipsychotics)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 19.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 18.
- Study Questions
 - Give 2 examples each of old and new generation neuroleptics.
 - Describe ADME for each.
 - What are some of the side effects of old and new generation neuroleptics?
 - What are some of the advantages of the new generation neuroleptics?

13.12 Antidepressants (MAO, TCA, SSRI)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 18.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 19.
- Study Questions
 - 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?
 - Compare and contrast mechanisms of action, ADME and side effects of TCAs, SSRIs and MAOs.

13.13 Anticonvulsants (Phenytoin, Carbamazepine, Valproic acid, Gabapentin, Lamotrigine, Topiramate)

- Required Literature Reading
 - Levine Principles of Forensic Toxicology, Ch 17.
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 21.
- Study Questions
 - Drugs used to control seizures have varied chemical structures. Describe each.
 - Describe the neurological pathways of seizure control.
 - Describe lethal toxicities associated with seizure medications.
 - Describe the metabolism of carbamazepine and its significance.
 - Describe the adverse effects of seizure medication on driving.
 - In OCME cases, what is the most important reason for the analysis of seizure medications?

13.14 Antihistamines/NSAIDS (Diphenhydramine, Promethazine, Dextromethorphan, ASA, APAP)

- Required Literature Reading
 - Goodman and Gilman's The Pharmacological Basis of Therapeutics, Ch 25, 27
 - NHTSA: Diphenhydramine, dextromethorphan
- Study Questions
 - Make a table of histamine receptors including localization within the body, antagonists associated with each and the therapeutic uses, therapeutic/toxic levels, therapeutic effects and effects on driving for each antagonist.
 - Why do antihistamines have anticholinergic effects?
 - Describe postmortem redistribution of antihistamines.
 - What antihistamines can be used in a DFSA? What screening method is used to detect them? What is their detection time in blood and urine?

14 DATA REVIEW AND CASE EXAMINATION

14.1 Objectives

- To learn the process and documentation involved in data review.
- To learn the process and documentation involved in case examination and technical review.
- To learn the process for creating and releasing cases using LIMS.

14.2 Methods of Instruction

- Data review and case examination training is primarily learned by observing multiple certified examiners and performing training examinations that are critiqued by certified examiners
- Trainee will observe and take notes of data (batch) review process with at least two experienced data reviewers.
- Trainee will observe and take notes of case examination and review process with at least two experienced examiners.
- Trainee will observe and take notes on LIMS Certificate of Analysis creation, technical review and release with at least two experienced examiners.
- Trainee will review the Toxicology Procedures Manual (Ch. 2), Quality Manual (Ch. 17.1). ASCLD/LAB Supplemental- Section 4.13 Control of Records, ISO 17025 – Section 4.13 Control of Records, Technical Review Form (Form 100-F111).

14.3 Laboratory Exercises

- Perform data review on alcohol, immunoassay, drug screen, GC quantitation, GCMS quantitation, LC-MS-MS quantitation batches with at least two different examiners.
- Perform case examinations on 10 non-IMPLIED consent cases with at least two different examiners (total 20 cases minimum). Cases should be a variety and include homicide, drug overdose, sexual assault (at least one), positive ethanol/drugs manslaughter, and decomposition cases. Medical examiner ethanol only cases are not included.
- Perform 10 DUID/DUI case examinations with at least two different toxicologists/examiners (total 20 cases minimum). No more than 5 ethanol only cases and 2 negative tox cases can be included in the 20.
- The trainee should document the review at least five case files using the appropriate Technical Review Form (TRF). Case files should be generated by multiple examiners, if possible. The potential findings of the reviews shall be discussed with the Training Coordinator. Technical Review Forms generated in this capacity shall be marked as Training and retained in their Training File. The case files shall be technically reviewed by a qualified examiner pursuant to QM 17 prior to release.

14.4 Evaluation

- Non-IMPLIED Consent cases. Trainer will select 20 cases that have not had final case examination performed. Trainee will perform final case examination using a Toxicology Summary Worksheet marked as a training case and submit cases to trainer for evaluation.

- DUID/DUI cases. Trainer will select 20 DUID/DUI cases that have not had final case examination performed. Trainee will perform final case examination using a Toxicology Summary Worksheet marked as a training case and submit cases to trainer for evaluation. Alternately, the data from released cases may be used.

14.5 Study Questions

- What does the analyst date on the batch chain-of-custody indicate?
- How many controls must be acceptable in a drug quantitative batch?
- How is carryover monitored in a drug quantitation? What is the appropriate response when carryover is detected?
- Describe occasions when a drug may be reported as present.
- An OCME case history lists rule out heroin. The blood morphine quantitation is 0.10 mg/L, 6AM none detected. As final case examiner, is this case complete? What other questions might you consider?
- A methadone quantitation was performed on femoral blood and heart blood. Would you expect the methadone concentrations to be different and if so why?
- A sexual assault case has immunoassay blood benzodiazepine negative, urine benzodiazepine pending and benzodiazepine quantitation none detected. As final case examiner is benzodiazepine testing complete?
- 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?

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15 UNCERTAINTY OF MEASUREMENT

15.1 Objectives

- To familiarize the trainee with traceability and its associated concepts.
- To familiarize the trainee with concepts of uncertainty of measurement.

15.2 Readings and Presentations

- Required
 - ASCLD/LAB Policy on Measurement Uncertainty (AL-PD-3060).
 - ASCLD/LAB Policy on Measurement Traceability (AL-PD-3057).
 - ASCLD/LAB Guidance on Measurement Traceability (AL-PD-3058).
 - ASCLD/LAB Guidance on Estimation of Measurement Uncertainty – Overview (AL-PD-3061).
 - ASCLD/LAB Guidance on Estimation of Measurement Uncertainty – ANNEX A: Details on the NIST 8 Step Process (AL-PD-3062).
 - ASCLD/LAB Guidance on Measurement Traceability – Measurement Assurance (AL-PD-3059).
 - ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D Toxicology Testing Discipline Example – Concentration of Ethanol in an Ante-Mortem Blood Specimen.
- Additional Resources
 - Presentations prepared by Dr. Wagner and DFS, available on the intranet.
 - Introducing the Concept of Uncertainty of Measurement in Testing in Association with the Application of the Standard ISO/IEC 17025 (ILAC-G17:2002).
 - Bell, S. A Beginner's Guide to Uncertainty of Measurement, Measurement Good Practice Guide No. 11 (Issue 2), ISSN 1368-6550.

15.3 Study Questions

- Define the following terms:
 - NIST
 - ASCLD/LAB
 - Mean
 - Median
 - Mode
 - Range
 - Accuracy
 - Precision
 - Gaussian distribution
 - Confidence Interval
 - Coverage Factor
 - Measurement

- Measurand
 - Type A evaluation
 - Type B evaluation
-
- Draw and explain what a Gaussian distribution is and how it relates to measurement uncertainty. Demonstrate two Gaussian distributions where one has high variability and one has low variability.
 - Obtain an uncertainty budget used in the toxicology section. Define the elements and from where the information is obtained.
 - Within the toxicology section, find a calibration standard that is traceable to NIST. Write a brief description of the traceability of that item.
 - Use the following information (Table 1) to calculate the expanded uncertainty at $k=2$ and $k=3$ and calculate the uncertainty of the measurement of alprazolam at 0.082mg/L. Report the measurement of alprazolam and its associated uncertainty at the 95.45% level of confidence.

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