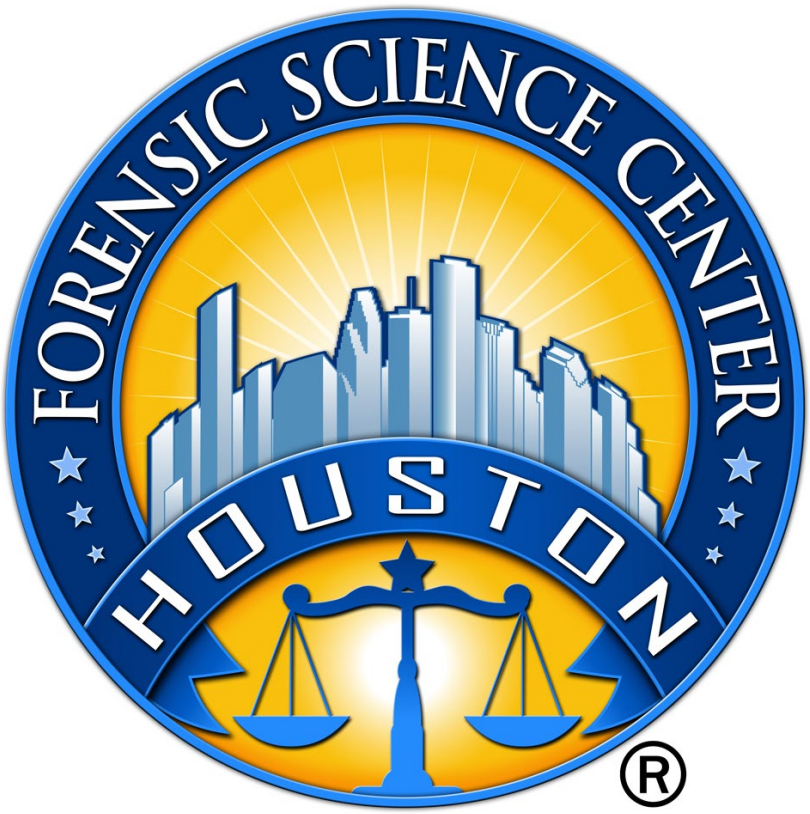

Forensic Biology
Biology/DNA Training Manual

Forensic Biology Division

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1. Overview of the Forensic Biology Training Program

1.1. Introduction

1.1.1. The purpose of this manual is to provide a training program for the analysis of forensic evidence by the Forensic Biology Section at the Houston Forensic Science Center. This program provides individuals with the theoretical background and the working knowledge and skills to conduct independent casework analysis and to provide expert witness testimony in the field of body fluid identification and DNA analysis for human identification.

1.1.2. Individuals employed by the Houston Forensic Science Center may function in different positions in the DNA section. There are three main categories of positions:

- Screeners/Evidence Processors
- DNA Technicians
- DNA Analysts

1.1.3. This training manual serves to educate and train those individuals serving as Screeners, DNA Technicians, and DNA Analysts.

1.1.4. A deficiency in one area can lead to the failure of an analysis and/or defense of the analysis in a court of law. Quality assurance guidelines must be followed for every sample processed in the training program.

1.1.5. The topics within this manual are organized into Modules, and the order of modules listed in this manual is not necessarily the order in which the training will be performed, though it is most often. It may be necessary to learn and perform some techniques out of order. Individuals may be trained in and obtain competency for individual techniques or processes. Individualized training plans, a training module checklist, and a required reading log, shall be provided to each trainee detailing the specific techniques/modules/articles in which training will be provided.

1.1.6. The Assessment Questions are not meant to be all encompassing. They are a reference to guide the trainees in their study and are an example of the type of questions that the trainee will be expected to be able to articulate both verbally and in writing, both technically and in laymen's terms for a jury.

1.1.6.1. All trainees will be expected to write out answers to the Assessment Questions and maintain them within their training binder. This exercise will be checked for completion but will not be individually graded.

1.1.7. Each trainee's profile must be on file for the DNA typing systems in use. The laboratory maintains a database of DNA profiles of personnel for the sole purposes of identification of the source of contamination and evaluation of in-house competency tests.

1.1.8. New screening/serological, DNA processing, data interpretation, or statistical analysis methods shall be incorporated into the laboratory's protocols and training manual. All personnel responsible for performing the new method shall successfully complete training and competency testing prior to performing DNA analysis, data interpretation or statistical analysis.

1.2. Requirements for Qualification

1.2.1. Prerequisites

1.2.1.1. Individuals must have a Bachelor's degree in Biology, Molecular Biology, Biochemistry, Genetics or a closely related field.

1.2.1.2. A DNA Analyst shall have successfully completed courses in biochemistry, genetics, molecular biology or other subjects providing a basic understanding of forensic DNA analysis, as well as course or training in statistics and/or population genetics as it applies to forensic DNA analysis.

1.2.1.3. A DNA Analyst shall have a minimum of six (6) months of forensic DNA laboratory experience, including the successful analysis of a range of samples typically encountered in forensic case work prior to independent case work analysis using DNA technology. This time may run concurrently with training.

1.2.1.4. DNA Technicians may be trained and obtain competency in individual Modules (7-11). Technicians do not interpret DNA data, reach conclusions on typing results, or prepare final DNA reports. The six months of required forensic DNA laboratory experience and the required coursework does not apply to technicians.

1.2.1.5. Screeners may be trained and obtain competency in individual Modules (1-6). Screeners do not interpret DNA data, reach conclusions on typing results, or prepare final DNA reports. The six months of required forensic DNA laboratory experience and the required coursework does not apply to screeners.

1.2.1.6. Training for trainees having prior experience in forensic serology and/or DNA procedures may be modified according to their skills and knowledge by the DNA Technical Leader. Individuals with documented previous experience and training in forensic DNA analysis may be exempted from portions of the training program. The Technical Leader must ensure the previous training is adequate and documented. The individualized training plan provided to the trainee will reflect the approved modification including the modules needed and the number of training sets within each module required.

1.2.1.7. A competency test is still required prior to independent participation in casework.

1.2.2. Exercises

1.2.2.1. Exercises are required to be completed in various training modules. They can include such topics as skills within the laboratory (i.e. assessing cross-reactivity and false positives, or pipetting skills, etc.), or practice data sets in DNA interpretation (i.e. inclusion vs exclusion).

1.2.2.2. Exercises are not graded but assessed for completion. An exercise can be repeated, at the discretion of the trainer, if the exercise as a whole is deemed unsuccessful.

1.2.2.3. Exercises are usually, but not always, followed by a quiz on the same topic material.

1.2.3. Quizzes

1.2.3.1. Quizzes examine a specific learning concept. They assess the trainee's knowledge of specific procedures and/or their understanding of the theoretical basis behind the learning concept.

1.2.3.2. A passing score of 80% is required on all quizzes which include written questions.

1.2.3.3. If a quiz does not have a passing score of at least 80%, remediation is provided to the trainee and a new quiz is administered. This new quiz can be provided in either a written or oral format. The questions and associated points will be documented prior to providing the new quiz to the trainee. Any additional points earned will be calculated with the original quizzes score.

1.2.3.4. A quiz is not a competency test.

1.2.4. Competency Test

1.2.4.1. A competency test is required prior to participation in casework. Participation will be allowed on only those areas that a competency test has been completed and been passed. If an analyst misses a proficiency cycle, he/she must complete a competency test prior to resuming casework.

1.2.4.2. A competency test(s) must include: a written, oral exam, and practical test (as applicable) in the areas in which the trainee is seeking to perform casework, and a written report (if the trainee is in a position that would issue reports). The competency test is designed to establish that the trainee has demonstrated achievement of technical skills and met minimum standards of knowledge necessary to perform forensic screening or DNA analysis.

1.2.4.3. Competency may only be assessed to the extent that an individual participates in casework (i.e. an individual may be found competent to review a methodology, but not to perform said methodology).

1.2.4.4. The DNA technical leader shall document approval of practical competency tests for the technician and analyst training plans prior to administration of the

test to the trainee. This approval shall be documented on the Practical Answer Key and Approval Form. The Technical Leader shall also evaluate and document the results of all competency tests. Competency test results shall be reviewed with the trainee, the trainer(s), and the trainee's supervisor.

1.2.4.5. Practical

1.2.4.5.1. 100% concordance with previously obtained results must be achieved on all practicals when the competency test consists of actual sample handling. Practicals for lab processes should be in the form of a case-type scenario.

1.2.4.5.2. If a practical test is not 100% concordant with previously obtained results, the trainer will provide remediation through the discussion of the laboratory technique(s) which produced non-concordant results. Additional practices may be assigned depending on the training module and trainer discretion. A new competency test will then be administered.

1.2.4.5.3. A passing practical for DNA Interpretation report writing training will have no more than 2 technical defects found during technical review. The trainer will provide remediation through the discussion of the technical defects and additional practices may be assigned. A new practical will then be administered.

1.2.4.6. Written Examinations

1.2.4.6.1. A passing score of 80% is required on all written exams. The written examination shall examine the trainee's knowledge of the procedures as well as the trainee's understanding of the theoretical basis for those procedures.

1.2.4.6.2. In addition, the trainee may be asked to demonstrate knowledge and understanding of quality assurance practices relevant to his or her work.

1.2.4.6.3. The exam shall not be discussed with, distributed or displayed to, or otherwise divulged to any other person without permission of the trainer.

1.2.4.6.4. If a written examination does not have a passing score of at least 80%, the trainer will restate the missed questions to the trainee in an oral format. Documentation of the questions asked, and associated points will be included in the trainee's binder. If the trainee can successfully articulate the answer, credit will be given. If the trainee cannot

successfully articulate the answer, additional review sessions and a new exam will be administered. Any additional points earned will be calculated with the original written examination score.

1.2.4.7. Oral Exams

1.2.4.7.1. All questions must be answered satisfactorily for oral exams.

1.2.4.7.2. If a question is not answered satisfactorily, the trainee will be administered a second oral exam that focuses solely on the missed question(s). If a question is not answered satisfactorily on the second oral exam, remedial training will be provided, and the oral exam will be re-administered.

1.2.5. Mock Trial

1.2.5.1. Please refer to the Quality Manual for the requirements of staff to complete a mock trial.

1.2.6. Licensing

1.2.6.1. Trainees must successfully obtain a forensic analyst, forensic technician, or biology screener license issued by the Texas Forensic Science Commission to begin processing casework.

1.2.7. Authorization Memo

1.2.7.1. The authorization memo will summarize the training documented within the training binder and outline specific methodology, equipment, and software that the trainee has completed.

1.2.7.2. All completed training activities and the authorization memo will be reviewed and approved by the Technical Leader and the Quality Division prior to the trainee beginning casework.

1.2.7.3. The approved authorization memo will be uploaded into the Quality Files for the trainee within Qualtrax.

1.2.8. Re-training of previously Qualified Laboratory Personnel

1.2.8.1. In the event that a previously qualified individual requires re-training, the DNA Technical Leader shall evaluate the knowledge-based or technical areas requiring improvement and determine the appropriate re-training and competency testing to

be completed. The individual shall successfully complete the re-training and assigned competency test(s) in order to resume the applicable job responsibilities.

1.2.9. Re-testing Within a Training Program

1.2.9.1. In the event that an individual needs to be re-tested to demonstrate competency, the DNA Technical Leader shall evaluate the knowledge-based or technical areas requiring improvement and determine the appropriate additional training necessary for the individual to demonstrate competency. Following completion of that training, the individual shall demonstrate competency in order to initiate or resume the applicable job responsibilities.

1.3. Assignment of Trainer

1.3.1. The DNA Technical Leader is responsible for the review and approval of the Training Program.

1.3.2. The FBIO Training Coordinator is responsible for overseeing all training in the Forensic Biology Section.

1.3.3. The FBIO Training Coordinator may assign a trainer(s) to the trainee for general laboratory assistance, for instruction in procedures, and for laboratory practicals and assessments.

1.3.3.1. Qualifications of Trainers

1.3.3.1.1. Trainers are qualified analysts and/or technicians who have been authorized to perform the tasks described in the training module.

1.3.3.2. The trainee and the trainer will meet regularly to evaluate the trainee's progress, plan future study/practical assignments and discuss any deficiencies, which may require additional training.

1.3.3.3. The trainer is responsible for creating, maintaining, and distributing training samples as well as records of their sources and known types. The amount and type of training samples is specified by each particular training module.

1.3.4. Pertinent scientific literature, SOPs, equipment manuals, and required readings shall be made available to the trainee. The trainee shall document the completion of these required readings in their Required Reading Log.

1.3.5. All potential safety hazards to the trainee must be explained by the trainer BEFORE performing a task that may involve that particular safety hazard.

1.3.6. It is acceptable for training to be administered by a qualified non-HFSC employee or an external party/vendor. For example, an Applied Biosystems Representative may conduct training on the Globalfiler PCR amplification kit, or a QIAGEN representative may perform

training on the QIAGEN EZ1 Advanced XL. External training for DNA analysis shall be pre-approved by the DNA Technical Leader. External training activities shall be documented in the trainee's training records. The documentation shall include the DNA Technical Leader's review of the external training.

1.3.7. For DNA personnel who had an integral role in the validation sufficient to master the technical skills, concepts and knowledge pertaining to the validation the technical leader may allow the validation to serve as the demonstration of competency. The level of involvement of the individual in the validation to indicate how it applies to the individual's job responsibilities will be documented on their Authorization Memo.

1.3.8. For personnel who had an integral role in the validation sufficient to master the technical skills, concepts and knowledge pertaining to the validation, the technical designee may allow the validation to serve as the competency test in this method of serological analysis. The technical designee shall document the level of involvement of the individual in the validation to indicate how it applies to the individual's job responsibilities.

1.4. Trainee Responsibilities

1.4.1. The trainee is required to keep up with the training program and reading assignments on a self-study basis. The trainee is responsible for informing his/her trainer or DNA Technical Leader when problems arise at any time during the training period. The trainee will examine the training samples according to the SOP, maintaining analysis documentation as if the training were casework.

1.4.2. Training Binder:

1.4.2.1. The trainee is required to keep a training binder that includes all work completed. The training binder will contain detailed records of his/her training, practice testing, and training progress. The following is a list of items maintained in the training binder by the trainee:

Required:

- Individualized Training Plan
- Training checklist recording the dates of all completed training activities
- Required Reading Log recording the dates of all completed readings
- Laboratory worksheets (Screeners/DNA Technicians) for all work completed
- Exercises from each module being trained in, if applicable
- Quizzes from each module being trained in, if applicable
- Written "Assessment Questions" from each module being trained in
- Qualifying tests and results
- Written exams from each module being trained in
- Competency Test Evaluation Form

As needed:

- Technical Review Checklists for completed cases
- Courtroom testimony attended and observations

1.4.2.2. Departures from approved laboratory protocol, as outlined within the Biology and/or DNA SOP, found within a trainee's practice sets will result in remediation of the training activity and the possible assignment of additional practice sets.

1.4.3. Casework authorization, including an authorization memo generated by the Technical Leader regarding the training activities included in the trainee's training binder, must be approved per the Quality Manual.

1.4.4. Retention of Training Records

1.4.4.1. The laboratory will maintain a copy of the completed training records of each trainee.

2. Module 1: Laboratory Introduction & Safety

2.1. Duration

2.1.1. One to two days

2.2. Purpose

2.2.1. To orient and acquaint the trainee with precautions and safety measures taken in the laboratory to prevent accidents.
2.2.2. To become aware of the potential hazards that a DNA analyst working in a forensic laboratory may encounter during the examination of biological specimens and the use of toxic materials.
2.2.3. Become familiar with this laboratory's method of disposing of hazardous materials related to DNA analysis.
2.2.4. Become familiar with the locations of safety equipment.
2.2.5. Locate the notebooks containing the laboratory's Safety Data Sheets (SDS). In addition, the SDS may be accessed online.

2.3. Prerequisites

2.3.1. None

2.4. Theoretical Objectives

2.4.1. Trainee will be able to:

- Describe the organization of the Houston Forensic Science Center
- Describe analyses performed in other HFSC Disciplines.
- Describe the potential hazards that exist in the laboratory.
- Discuss the necessity for detailed comprehensive notes and adequate labeling of evidential materials.
- Attend Firearms Safety and Unloading Training conducted by the Firearms Section

2.5. Practical Skills

2.5.1. Trainee will be able to:

- Don and doff the appropriate PPE for Evidence Processing, Pre-PCR, and Post-PCR laboratory settings.
- Locate first aid kits, safety showers, and eye washes.
- Properly use the safety shower and eyes washes in the event of an emergency.
- Describe the fire evacuation plan for HFSC.
- Properly handle and unload firearms in a safe manner.

2.6. Module Outline

I. Infectious Agents • Viral Agents, including HIV and Hepatitis

- Bacteria, including sexually transmitted diseases
- Fungi
- Parasites

II. Hazardous Material
- Caustic Agents (Acids and Bases)
- Carcinogens/Mutagens
- Organic Chemicals

III. Electrical Hazards
- Shock from any pieces of electrical equipment

IV. Burn Hazards
- Autoclaves
- Hot Plates

IV. Eye Damage
- Alternate light source
- Ultraviolet (UV) light

2.7. Theory

2.7.1. The trainee must be trained in laboratory safety. Various manuals are provided that must be followed to ensure safety of all laboratory personnel. The following manuals are to be used for reference and guidance for laboratory safety: Reagent SDS readings and the Houston Forensic Science Center Health and Safety Manual. The trainee will also be briefed on the fire evacuation plan for the laboratory as well as blood borne pathogen safety. Additional material (i.e. evacuation plans, chemical inventory, PPE policy, etc.) is available in Qualtrax under: Documents/Corporate Policies and Procedures/Health and Safety Documents.

2.7.2. It is the responsibility of the trainer to alert the trainee to safety hazards specific to the laboratory.

2.7.3. The trainee will be taken throughout the laboratory and shown areas of interest to his/her work.

2.7.4. Personal Protective Equipment (PPE) must be worn at all times while handling samples. This is to protect both the analyst and the sample. Appropriate PPE includes lab coat, gloves, hairnet, and mask in Pre-PCR laboratories, and lab coat and gloves in Post-PCR laboratories. Safety glasses or goggles may be required during some operations.

2.7.5. Use extreme caution when operating autoclaves. Do not open autoclave until pressure reads zero and it has cooled to safe handling temperature.

2.7.6. Assessment Questions
- Are there clothing requirements related to laboratory safety? Are they different between Pre- and Post?

- How are biohazards disposed of in the DNA laboratory?
- List the safety equipment available in the DNA laboratory and where is it located.
- What safety hazards are associated with the DNA Investigator kits?
- What safety hazards are associated with the Prepfiler kits?
- How are the EZ1 supplies discarded? The Prepfiler reagents?
- Describe how to operate the safety shower? Both eye wash stations?
- If you cut yourself, what steps would you take? Where is the location of the closest First Aid kit to your desk? The closest fire extinguisher?

2.8. Readings

2.8.1. See Required Reading Log – Module 1: Laboratory Introduction & Safety

2.9. Practice Exercises

2.9.1. None

3. Module 2: Evidence Handling

3.1. Duration

3.1.1. One to two days

3.2. Purpose

3.2.1. To orient and acquaint the trainee with precautions taken in the laboratory to prevent contamination and loss of DNA.

3.2.2. To gain familiarity with the following:

- Collection, packaging, handling, and storage of evidence
- Chain of Custody
- Case and evidence acceptance policy
- Laboratory Information Management System (LIMS) as it relates to case triage and chain of custody documentation
- Policies regarding consumption of evidence (refer to the Quality Manual)
- Laboratory abbreviations, itemizations, sub-itemizations, and electronic files
- Laboratory case files

3.3. Prerequisite

3.3.1. Module 1: Laboratory Introduction & Safety

3.4. Theoretical Objectives

3.4.1. Trainee will be able to:

- Describe factors and conditions that influence the deterioration of evidence as it relates to packaging, handling, and storage conditions and time.
- Describe Evidence Handling Procedures regarding receiving, identifying, and handling of evidence, as well as specific guidelines for handling biological evidence in the Forensic Biology Section.
- Describe chemicals and environmental insults promoting the degradation and loss of DNA.
- Describe actions taken to preserve DNA.
- Describe the possible sources of contamination in the laboratory.
- Describe the necessary precautions taken to prevent contamination within each dedicated work area.
- Discuss the effects of contamination on casework samples.
- Understand when it is appropriate to collect contact DNA or perform chemical testing for the presence of bodily fluids, or both.
- To develop a basic understanding of the sexual assault evidence collection kit, including common and uncommon contents, the hospital report contained within, when to test which items, and when to portion for DNA analysis.

3.5. Practical Skills

3.5.1. Trainee will be able to:

- Prepare a 1:10 bleach solution and properly clean work surfaces
- Properly select the correct cleaning process and/or solution for DNA equipment and supplies such as pipettes, centrifuges, heat blocks, thermomixers, reagent bottles and the thermal cycler
- Properly sterilize the necessary supplies, reagents, and dH_2O
- Properly cleanse forceps, scissors and other tools
- Demonstrate proper handling of samples, handling of microcentrifuge tubes, proper pipetting techniques, and use of reagents
- Swab items for possible contact DNA
- Properly portion evidence for further testing
- Properly dispose of used or contaminated materials and supplies

3.6. Module Outline

I. DNA Degradation
- Chemicals, detergents
- UV light, sunlight
- Heat, humidity
- Bacteria, microorganisms
- Endo and exonucleases
- Biological fluid mixtures
- Shear forces
- Time

II. Preserving stains/DNA
- Drying
- Refrigeration or freezing
- EDTA additive

III. Sources of laboratory contamination
- Human genomic DNA from the environment
- Sample to sample contamination
- PCR product carry-over

IV. Contamination prevention measure

A. Isolation and dedicated work areas
- Evidence handling work area
- DNA extraction work area
- PCR set-up work area
- Amplified DNA work area

V. Special Precautions
- Dedicated equipment and lab supplies
- Clean work surfaces and equipment

- Clean cutting surface
- Frequent glove changes
- Cleansed scissors or disposable blades, forceps
- Disposable pipette tips
- Minimizing aerosols
- Avoiding splashes
- Autoclaving, sterilization
- UV germicidal lamps
- Separating evidence from reference samples
- Single sample handling
- Small sample size and/or number
- Aliquot reagents

VI. Sexual Assault Kits

- Standard Contents
- Using the hospital report as a guide to determine the appropriate analysis and verifying the items collected are contained within the kit.

3.7. Theory

3.7.1. The DNA Unit uses Polymerase Chain Reaction (PCR) Technology, which allows very small amounts of DNA to be amplified over a million times. Because of the sensitivity of this technique, contamination control is a very serious issue that must be emphasized and practiced with every sample, starting with the identification of the fluids present on evidence. The Evidence Handling Procedures must be strictly followed.

3.7.2. Proper PPE must be worn at all times in all laboratory areas. This is to protect both the analyst and the samples.

3.7.3. The issue of contamination and loss of DNA in the laboratory is of paramount concern to everyone working in serology/DNA and to the legal system. With PCR DNA methods, a trace amount of contaminant DNA could be detrimental to the outcome of an analysis. Therefore, stringent measures and precautions are practiced to prevent and minimize contamination.

3.7.4. Equipment cleaning schedule as noted in the DNA SOP. Documentation and use of required forms when cleaning specific equipment.

3.7.5. Trainer will demonstrate proper procedures for cleaning and preparing work surfaces and equipment in the dedicated work areas. Trainer will give instruction on the cleansing of forceps and cutting tools, frequent changing of gloves and separation of evidence. Trainer will give instructions on the supplies and reagents requiring sterilization. Trainer will demonstrate proper handling of single samples, decapping microcentrifuge tubes, pipetting techniques and handling of reagents. Trainer will give instruction on the

collection of contact DNA and sample portioning. Trainer will give instruction on the disposal of used and contaminated supplies.

3.7.6. Assessment Questions:

- Discuss the precautions taken in the amplified DNA work areas.
- What is considered an acceptable seal for evidence being received?
- If an analyst notices a discrepancy upon opening evidence, how should they proceed?
- How should perishable evidence be stored?
- What is considered contact DNA?
- What type of evidence is considered inappropriate for testing?
- What is the purpose of the 10% bleach solution or DNA Away when cleaning tools?
- What is the purpose of Ethanol during cleaning?
- How do you handle evidence that is still wet upon receipt?
- You examine multiple items of evidence, one after the other on the same day. You set out a clean piece of paper to examine the first. Would you change the paper before examining the second? Explain.
- Describe the use of and reason behind the staff elimination database? Who all is represented in that database?
- When is permission to consume an item of evidence required to be obtained?
- Contamination Scenarios: Describe specific case circumstances when the following ways of contaminating biological evidence would be possible and realistic. Then explain how to prevent that contamination.
  - Contamination from another piece of evidence
  - Contamination from the surface it's examined on
  - Contamination from the tools it's examined with
  - Contamination from the reagents or reagent containers used
  - Contamination from the analyst who is examining it
  - Contamination from the analyst's personal protective equipment (PPE)
  - Contamination from other individuals who are present during collection or analysis

3.8. Readings

3.8.1. See Required Reading Log – Module 2: Evidence Handling

3.9. Practice Exercises

3.9.1. Prepare a 1:10 bleach solution and clean/prepare a work area in the screening area or the extraction room.

3.9.2. Properly clean a centrifuge using the appropriate technique and cleaning supplies.

3.9.3. Demonstrate proper laboratory techniques to minimize contamination. Techniques being evaluated include: handling & labeling tubes, portioning cloth & swabs, swabbing for contact DNA, and pipetting various volumes. The following will be done in order.

- Trainee will be given 5 unstained pieces of cloth, each inside a separate envelope.
- Trainee will be given 5 unused swabs and asked to swab 5 items for the presence of possible contact DNA.
- Trainee will label 10 microcentrifuge tubes (1 through 10) and place them in a rack.
- Trainee will be observed cutting 1/2 cm squares from each of the cloth samples and placing them into the appropriate tubes.
- Trainee will be observed portioning approximately ½ of the swabs and placing them into the appropriate tubes.
- Trainee will be observed pipetting 1000 microliters into each tube.
- Trainee will then be observed pipetting 10 microliters into each tube.

4. Module 3: Identification of Blood and Species Origin

4.1. Duration

4.1.1. One to two weeks

4.2. Purpose

4.2.1. To develop a basic understanding of the methodology and theory of chemical, microscopic, and immunological testing procedures used to identify blood and determine human origin.
4.2.2. To know the mechanism by which the phenolphthalein reaction and the Hematrace reaction occur.
4.2.3. Understand the sensitivity and the specificity of the phenolphthalein test and Hematrace.
4.2.4. Understand the QC of presumptive blood testing reagents and their associated controls.

4.3. Prerequisite

4.3.1. Module 2: Evidence Handling
4.3.2. May be done simultaneously or prior to Module 4: Identification of Semen

4.4. Theoretical Objectives

4.4.1. To understand the chemical reactions involved in phenolphthalein test
4.4.2. To understand the principle of immunology involved in antigen-antibody reactions.

4.5. Practical Skills

4.5.1. Trainee will be able to:

- Perform presumptive phenolphthalein test.
- Describe a positive and negative result for phenolphthalein test.
- Describe false positives and possible sources of false positives for phenolphthalein.
- Perform ABACard Hematrace test, if applicable to analyst's training.
- Describe a positive and negative result for ABACard Hematrace test, if applicable.

4.6. Module Outline

I. Presumptive tests for the presence of blood
   - Catalytic color tests
   - Sensitivity
   - Specificity
   - False positives
   - Representative testing

II. Confirmatory tests for the presence of blood (Optional)
   - Immunological tests
   - Sensitivity

- Specificity
- False positives
- High-dose hook effect

4.7. Theory

4.7.1. The Forensic Biology section screens evidence for the presence of blood. Visual examinations can identify areas for the chemical testing of blood. Items that screen positive for blood may be submitted for human identification DNA analysis.

4.7.2. Assessment Questions

- What are the components of blood? Which components are important to modern forensic science?
- Which cells in blood contain nuclear DNA? What percentage of that cell type is present in whole blood?
- Describe how blood might look on different colored backgrounds (e.g. dark, light, etc.) and if deposited in different concentrations (e.g. concentrated blood stain vs. light or dilute deposit)
- What tests are used during evidence processing to test for the presence of blood?
- What structure in blood reacts with the catalytic reagents of the phenolphthalein test resulting in a positive reaction? Describe the mechanism by which the reaction occurs?
- Why should the reaction between the stain and the phenolphthalein reagents be read immediately?
- What are other names for the phenolphthalein test?
- What is the reported sensitivity of phenolphthalein?
- What sensitivity of phenolphthalein testing did you obtain on your serial dilution of human blood testing?
- According to Cox, what types of fabrics (that were bloodstained) will still give a positive result with a phenolphthalein test even after they were washed? Were the stains visible after washing?
- What controls must be tested and how must they be documented before using phenolphthalein testing in casework?

4.8. Readings

4.8.1. See Required Reading Log – Module 3: Identification of Blood and Species Origin

4.9. Practice

4.9.1. Demonstration by trainer and/or supervised performance

4.9.1.1. Trainer will demonstrate the presumptive methods being introduced.

4.9.1.2. Trainer will discuss proper documentation and QC of reagents.

4.9.2. False Positive Phenolphthalein Exercise
4.9.2.1. Test a variety of substances and biological fluids on varying fabrics.

4.9.3. Sensitivity of Phenolphthalein Exercise
4.9.3.1. Perform serial dilutions up to 1:100,000 of human blood to determine sensitivity of phenolphthalein.

4.9.4. LEEDS Spectral Vision System (LSV2) ALS Practice
4.9.4.1. Examine a minimum of four samples for blood using the LSV2.

4.9.5. (Optional) Cross-reactivity of Phenolphthalein vs Hematrace Exercise
4.9.5.1. Test phenolphthalein and the ABAcard Hematrace test for cross reactivity using blood from the following animals: Dog, Cat, Cow, Deer, and Fish.

4.9.6. (Optional) Sensitivity of ABAcard Hematrace
4.9.6.1. Perform serial dilutions up to 1:1,000,000 of human blood to determine sensitivity of the ABAcard Hematrace test.

4.9.7. Practice Sets – Laboratory Skills
4.9.7.1. Process a minimum of four mock cases, or comparable blood samples
4.9.7.2. These can be a combined practice sets for the blood and semen identification modules.

4.10. Conclusion

4.10.1. Blood Quiz
4.10.1.1. A quiz on blood will be administered.
4.10.1.2. A hardcopy of the quiz will be stored within the training binder.

4.10.2. Competency Test
4.10.2.1. Practical
4.10.2.1.1. A practical consisting of at least one Sexual Assault Kit (SAK) or comparable blood samples, will be administered.
4.10.2.1.2. A practical will be administered for the LEEDS Spectral Vision System (LSV2) and ABAcard Hematrace when applicable.
4.10.2.1.3. This can be a combined practical for the blood and semen identification modules.

4.10.2.2. Written Exam
4.10.2.2.1. A written presumptive blood test will be administered.
4.10.2.2.2. A written test will be administered for the LEEDS Spectral Vision System (LSV2) and ABAcard Hematrace when applicable.
4.10.2.2.3. This can be a combined serology exam for both the blood and semen identification modules.
4.10.2.2.4. A hardcopy of the exam(s) will be stored within the training binder.

4.10.2.3. Oral Exam/Mock Trial

4.10.2.3.1. Successful completion of a mock trial is required. Request for the completion of an Oral Exam at Technical Lead Discretion.

5. Module 4: Identification of Semen

5.1. Duration

5.1.1. One to two weeks

5.2. Purpose

5.2.1. To understand the methodology and theory of testing procedures used to identify semen.
5.2.2. To learn how different biological materials will appear with an Alternate Light Source (ALS) and how stains are marked/documented on evidence when they are located with an ALS.
5.2.3. Understand what other commonly encountered materials will also fluoresce with an ALS.
5.2.4. Understand the QC of presumptive semen testing reagents and their associated controls.

5.3. Prerequisite

5.3.1. Module 2: Contamination and Evidence Handling
5.3.2. May be done simultaneously or prior to Module 3: Identification of Blood and Human Origin

5.4. Theoretical Objectives

5.4.1. To develop a basic understanding of the methodology and theory of chemical, microscopic, and immunological testing procedures used in the identification of semen.
5.4.2. To understand and identify sperm cell structure and morphology.

5.5. Practical Skills

5.5.1. Trainee will be able to:

- Screen for bodily fluids utilizing the Alternate Light Source (ALS).
- Describe positive and negative results for the Alternate Light Source.
- Perform presumptive Acid Phosphatase Spot tests.
- Describe a positive and negative result for Acid Phosphatase Spot tests
- Develop a working knowledge of the stereo and compound microscopes tests

5.6. Module Outline

I. Presumptive tests for the presence of semen
   - Catalytic color tests
   - Immunological tests
   - Sensitivity
   - Specificity
   - False positives

II. Confirmatory tests for the presence of semen (Optional)
   - Microscopy

5.7. Theory

5.7.1. The Forensic Biology section screens evidence for the presence of semen using many tools, including both presumptive and confirmatory tests. Presumptive tests include the alternate light source, acid phosphatase testing, and prostate specific antigen (PSA); microscopy can be used as a confirmatory test. Items that screen positive for the presence of semen may be further screened for the presence of male DNA, using qPCR and submitted for human identification DNA analysis.

5.7.2. Sexual assault offenses usually warrant testing for the presence of semen. However, it is not uncommon for non-sperm DNA to be probative in sexual assault cases.

5.7.3. Assessment Questions

- Describe the general contents of a Sexual Assault Kit (SAK).
- Describe sperm cell morphology.
- What is the composition of semen? How are these components important to the identification of semen in forensic casework? Which components might be useful for DNA analysis?
- What is the approximate volume of an average human ejaculation? How many spermatozoa are present, on average?
- Approximately how much DNA is contained in a sperm head?
- Can seminal acid phosphatase be detected on clothing (using the AP test) after it has been washed? Explain.
- Can a semen stain give a negative AP test? Why or why not?
- What is p30? How is it different from acid phosphatase?
- What body fluids are expected to fluoresce with an ALS? Which ones are not?
- Does semen always fluoresce with an alternate light source? Why or why not?
- What are some non-biological fluids that may fluoresce.
- What combination of wavelength and colored glasses work best for locating seminal fluid stains?
- Why might it be difficult or impossible to see fluorescing stains on dark substrates such as black fabric?
- In what kinds of sexual assault offenses would semen not be probative?

5.8. Readings

5.8.1. See Required Reading Log – Module 4: Identification of Semen

5.9. Practice

5.9.1. Demonstration by trainer and/or supervised performance

5.9.1.1. Trainer will demonstrate the presumptive methods being introduced.

5.9.1.2. Trainer will discuss proper documentation and QC of reagents.

5.9.2. Alternate Light Source Exercise
5.9.2.1. Examine blood, semen, saliva, and sweat using the alternate light source. Substrates shall include several fabric and materials often encountered in casework.

5.9.3. LEEDS Spectral Vision System ALS Practice (LSV2)
5.9.3.1. Examine a minimum of four samples for semen using the LSV2.

5.9.4. CrimeScope CS-16-500
5.9.4.1. Examine a minimum of four samples for semen using the LSV2.

5.9.5. False positive Acid Phosphatase Exercise
5.9.5.1. Test a variety of substances and biological fluids on varying fabrics.

5.9.6. Sensitivity of Acid Phosphatase Exercise (Optional: Seratec PSA (p30) test)
5.9.6.1. Perform serial dilutions up to 1:100,000 of semen to determine limits of sensitivity with acid phosphatase and the Seratec PSA (p30) test.

5.9.7. (Optional) Cross-reactivity of Seratec PSA (p30) Exercise
5.9.7.1. Test human semen, blood, urine, saliva, feces, and semen free vaginal swabs for p30 using the Seratec PSA (p30) test.

5.9.8. Practice Sets – Laboratory Skills
5.9.8.1. Observation of SAK and Non-SAK Casework
5.9.8.1.1. The analyst will observe analysis of at least ten SAK and non-SAK cases.
5.9.8.1.2. The observed cases must include the following items:
- A morgue kit
- An article of clothing
- An item with a pending latent print request

5.9.9. General SAK and Non-SAK Casework
5.9.9.1. The analyst will process a minimum of four non-SAK cases and generate a screening report. Performance will be evaluated, and additional mock cases will be provided as needed.
5.9.9.2. The analyst will process a minimum of three mock SAKs or comparable semen samples. Performance will be evaluated, and additional mock cases will be provided as needed.
5.9.9.3. These can be a combined practice sets for the blood and semen modules.

5.9.10. (Optional) Microscopic Examination of Animal Spermatazoa
5.9.10.1. Examine spermatozoa from various animals and document observations.

5.9.11. (Optional) Microscopic Examination of Human Spermatazoa
5.9.11.1. Perform serial dilutions up to 1:100,000 of semen and examine spermatozoa using SERI Christmas Tree stain.

5.10. Conclusion

5.10.1. Semen Quiz

5.10.1.1. A quiz on semen will be administered by the trainer.

5.10.1.2. A hardcopy of the quiz will be stored within the training binder.

5.10.2. Competency Test

5.10.2.1. Practical

5.10.2.1.1. A practical test consisting of at least one Sexual Assault Kit (SAK) or comparable semen samples will be administered.

5.10.2.1.2. A practical test will be administered for the LEEDS Spectral Vision System (LSV2), CrimeScope CS-16-500, SERAtec PSA (p30), and Microscopic Spermatozoa Examination when applicable.

5.10.2.1.3. This can be a combined practical test for the blood and semen identification modules.

5.10.2.2. Written Exam

5.10.2.2.1. A written presumptive semen test will be administered.

5.10.2.2.2. A written test will be administered for the LEEDS Spectral Vision System (LSV2), CrimeScope CS-16-500, SERAtec PSA (p30), and Microscopic Spermatozoa Examination when applicable.

5.10.2.2.3. This can be a combined serology exam for both the blood and semen identification modules.

5.10.2.2.4. A hardcopy of the exam(s) will be stored within the training binder.

5.10.2.3. Oral Exam/Mock Trial

5.10.2.3.1. Successful completion of a mock trial is required. Request for the completion of an Oral Exam at Technical Lead Discretion.

6. Module 5: Screening Case Mentorship Program

6.1. Duration

6.1.1. Minimum of two months

6.2. Purpose

6.2.1. Once the trainee completes his/her serological competency tests, the trainee is placed in a case mentorship program under experienced serologists. The purpose of this mentorship is to allow the newly qualified analyst to work cases under the close supervision of an experienced analyst. As techniques are added to an experienced analyst's repertoire, he/she is not required to reenter the case mentorship program.

6.3. Prerequisite

6.3.1. Module 3: Identification of Blood and Human Origin
6.3.2. Module 4: Identification of Semen

6.4. Theoretical Objectives

6.4.1. Gain experience with a variety of SAK and non-SAK case evidence and the application of presumptive blood and semen testing to that evidence.

6.5. Practical Skills

6.5.1. Trainee will be able to:

- Enter case and sample information into LIMS
- Perform presumptive blood and semen testing on different types of SAK and non-SAK evidentiary items.

6.6. Module Outline

6.6.1. Entering sample/case information into LIMS
6.6.2. Entering screening results into LIMS
6.6.2.1. Identifying discrepant or case-specific notes that should be included in reports, such as previous handling by another HFSC discipline

6.7. Theory

6.7.1. Assessment Questions

- From where would a DNA sample be collected from a bottle? How is this achieved?
- What size cuttings from an envelope flap would be targeted?
- What component in blue jeans can cause inhibition?
- How can inhibitors be decreased at the sampling step?

- Describe the process for preserving and documenting observed apparent hairs/fibers on an item of evidence.
- What steps are required when a firearm is received in FBIO for testing?

Evidence Scenarios:

- An officer requests DNA to be collected on an item already processed for latent prints. They also have an unprocessed cigarette butt. What do you tell them?
- An officer thinks a burglary suspect walked out the back door of a home, leaving blood on the handle. They swabbed it for DNA. Will we process this item for the suspect's DNA?
- During a robbery, the victim's car was stolen. It was recovered shortly after and they swabbed the steering wheel. Does the requesting officer have to submit standards from the victim?
- The complainant has no recollection from the night before. There is underwear in the sexual assault kit. How will the underwear be processed for semen?
- The medical report noted menstruation during the exam. A tampon and underwear were found in the sexual assault kit. Will we send phenolphthalein positive samples to DNA?

6.8. Readings

6.8.1. See Required Reading Log – Module 5: Screening Case Mentorship

6.9. Practice

6.9.1. Supervised SAK and Non-SAK Casework

6.9.1.1. The newly qualified analyst will process a minimum of 10 non-SAK cases and 10 SAK cases under the supervision of an experienced analyst. Performance will be evaluated, and additional supervised cases will be provided as needed.

6.9.3 The newly qualified analyst must have their first 25 cases technically reviewed by a supervisor or FBIO management approved analyst.

6.9.3 SAK and Non-SAK Technical Reviews

6.9.3.1 Analysts will observe a senior analyst perform two technical reviews of SAK cases and two technical reviews of non-SAK cases. Training for SAK and non-SAK technical reviews may occur at different time periods.

6.9.3.2 Analysts will perform technical reviews on a minimum of 10 SAK cases and 10 non-SAK cases, after which an experienced analyst will perform a second technical review and provide written feedback to the analyst. Additional technical reviewing opportunities will be provided as needed for the analyst to demonstrate proficiency in this area.

6.10 Conclusion

6.10.3 The trainee will be authorized to perform independent casework via a signed authorization memo.

6.10.4 The newly qualified analyst may be signed off to perform casework analysis, write screening reports, and technically review cases at separate times.

6.10.5 Non-SAK training can be performed in combination with the Identification of Semen and Blood training.

7 Module 6: Courtroom Testimony

7.9 Duration

7.9.3 One to two weeks

7.10 Purpose

7.10.3 To develop the skills necessary to effectively provide expert testimony about testing results in a court of law.

7.11 Prerequisites

7.11.3 None

7.12 Theoretical Objectives

7.12.3 Develop a working knowledge of the terminology and the presentation of analysis and results.
7.12.4 Express written and oral results simply, concisely, and accurately.

7.13 Practical Skills

7.13.3 Trainee will be able to:

- Verbally present results of testing both technically and non-technically, as if to a jury.

7.14 Theory

7.14.3 Results of analysis must be presented simply, concisely, and accurately in court.
7.14.4 Analysts must be capable of presenting findings of analysis both technically and non-technically, as if to a jury.

7.14.5 Example Assessment Questions

- What is accreditation?
- Is HFSC accredited? If so, by whom and how often?
- Describe the proficiency test obligations?
- What is an amended report?
- What information would you want to convey to qualify you as an expert on the stand?

7.15 Readings

7.15.3 See Required Reading Log – Module 6: Courtroom Testimony

7.16 Practice

7.16.3 (Optional) Court Room Observation

7.16.3.1 Attend court and observe experienced analysts testify.

7.17 Conclusion

7.17.3 Oral Exam/Mock Trial

7.17.3.1 Request for the completion of an Oral Exam and/or Mock Trial at Technical Lead discretion.

8 Module 7: Introduction to DNA - History of DNA

8.9 Duration

8.9.3 One to two days

8.10 Purpose

8.10.3 Enable a trainee to communicate with appropriate forensic DNA terms.
8.10.4 Educate a trainee on the earlier procedures and advances in forensic DNA.

8.11 Prerequisite

8.11.3 Trainee must have an individualized DNA Technician Training Plan

8.12 Theoretical Objectives

8.12.3 Define and understand basic genetic, heredity, and forensic DNA typing terms.
8.12.4 Discuss from a historical point of view the major developments in forensic DNA.
8.12.5 Describe the limitations of DNA analysis.

8.13 Practical Skills

8.13.3 None.

8.14 Module Outline

I. Introduction to forensic DNA
- Definition, importance, legal value
- The scientific basis of DNA typing
- Other areas utilizing DNA typing techniques

II. Overview of forensic DNA typing systems
- RFLP
- PCR - HLA Dqa and D1S80
- PCR – STR
- Mitochondrial DNA

III. Procedures for forensic DNA analysis
- Isolation of DNA
- Determining quality and quantity of DNA
- PCR amplification
- Analysis of PCR product

8.15 Theory

8.15.3 The ability to understand and use proper biological terminology is necessary for successful communications in both the forensic field and in the courtroom. The history of the

development and past methods in human identification testing will provide the trainee with a foundation of the concepts and the procedures used in the laboratory for forensic DNA testing.

8.15.4 Assessment Questions

- Define alleles.
- What steps are taken to prevent contamination in the DNA portion of the laboratory?
- How can contamination be detected? At what stage is it most likely introduced?
- Why are STRs useful for human identification?
- Explain the basics of heredity.
- Why is PCR prone to contamination?
- What are the major milestones in the advancement of DNA testing techniques/technology? Explain each briefly.
- What is mitochondrial DNA? How does it differ than autosomal DNA?
- What is Y-chromosomal DNA?

8.16 Readings

8.16.3 See Required Reading Log – Module 7: Introduction to DNA and DNA History

8.17 Practice

8.17.3 Pipetting Exercise

8.17.3.1 A pipetting skills session to demonstrate proper technique for pipette function, liquid aspiration, and liquid dispensing of various volumes of DNA using the different brands of pipettes available within the laboratory.

9 Module 8: DNA Extraction

9.9 Duration

9.9.3 Approximately eight weeks

9.10 Purpose

9.10.3 Enable trainee to recover and isolate DNA from a variety of forensic specimens
9.10.4 To become familiar with the operation of the EZ1 Advanced XL, QIAcube, and the Hamilton STAR and STARlet instruments.
9.10.5 To become familiar with the worksheets used and the documentation of reagent lot numbers and critical equipment.
9.10.6 To learn the purpose of each reagent used in the extractions.
9.10.7 To develop an understanding of the theory and practice of differential lysis for the separation of sperm DNA and epithelial cell DNA.
9.10.8 To become familiar with the limitations of the extraction procedure and to know which procedure to use for each evidence type (differential, high level, low level, and reference).
9.10.9 To become proficient with the use of and maintenance of pipettes, centrifuges, thermomixers and other equipment used in the DNA extraction process.

9.11 Prerequisite

9.11.3 Module 2: Evidence Handling
9.11.4 Module 7: Introduction to DNA and DNA History
9.11.5 May be done simultaneously with or prior to Module 9: DNA Quantification, Module 10: DNA Amplification, and Module 11: Capillary Electrophoresis

9.12 Theoretical Objectives

9.12.3 Trainee will be able to:

- Describe and become familiar with the various extraction methods and purification techniques used on forensic specimens.
- Learn the purpose and benefits of the various DNA extraction methods performed in the laboratory.
- Describe techniques to increase the yield of DNA and to remove inhibitors of PCR amplification.
- Understand the quality control and precautionary measures associated with the DNA extraction methods utilized in the laboratory.

9.13 Practical Skills

9.13.3 Trainee will be able to:

- Prepare and store the necessary reagents used in DNA extractions.
- Perform the necessary precautions to prevent contamination during extraction procedures.
- Isolate DNA using one or more of the following:

- Non-differential extraction using the EZ1 DNA Investigator kit on the QIAGEN EZ1 Advanced XL
- Differential lysis extraction of semen stains via QIAGEN QIAcube workstation and QIAGEN EZ1 Advanced XL
- Non-differential extraction using the Prepfiler Forensic DNA Kit on the Hamilton Microlab ID STARlet
- Differential extraction using the QIAGEN Investigator Lyse & Prep Kit on the Hamilton STAR

- Perform and document the necessary quality control measures practiced during extractions.

9.14 Module Outline

I. Contamination prevention measures
- Review of contamination control in DNA work area

II. Isolating DNA by EZ1 DNA Investigator Kit
A. Purpose, benefits
B. Methods
- Non-differential or straight extraction (high or low level)
- Reference extraction
- Differential extraction
- Hair root extraction

III. QIAGEN EZ1 Advanced XLs
- Operation
- Maintenance (daily, weekly, monthly, annual, performance checks, etc.)
- Sample Set-Up (kit components)

IV. QIAGEN QIAcube Workstation
- Operation
- Maintenance (daily, weekly, monthly, annual, performance checks, etc.)
- Sample Set-Up (kit components)

V. Isolating DNA by Prepfiler Forensic DNA Kit
A. Purpose, benefits
B. Methods
- Non-differential or straight extraction (high or low level)
- Reference extraction

VI. Hamilton STAR & Hamilton Microlab ID Starlet
- Operation
- Maintenance (daily, weekly, monthly, annual, performance checks, etc.)
- Sample Set-Up (kit components)

VI. Quality Control
- Forms, worksheet documentation
- Critical reagents

- Reagent blanks
- Separate extraction of known and questioned samples
- Sample controls (hair)

VII. Preserving extracted DNA

9.15 Theory

9.15.3 Successful PCR amplification relies upon the isolation and purification of genomic DNA from forensic samples. These extraction methods and purification techniques recover DNA and eliminate or minimize PCR amplification inhibitors arising from environmental insults and/or from substrates. The sensitivity of PCR renders evidentiary samples, particularly those with small amounts of DNA, at risk to laboratory contamination. Therefore, stringent precautionary measures and quality control are practiced during extraction procedures.

9.15.4 The ability to separate epithelial and sperm DNA from mixed samples through differential extractions provides for meaningful interpretation of DNA profiles in sexual assault reporting and CODIS entry.

9.15.5 Assessment Questions

- What safety hazards are associated with the DNA Investigator kits?
- What safety hazards are associated with the Prepfiler kits?
- What are some advantages of using automation in the laboratory?
- How are the EZ1 supplies discarded? The Prepfiler reagents?
- Explain the three main steps of an extraction. What is the purpose of each step?
- Describe how the EZ1 robots extract DNA from cells? What is the purpose of the digest buffer/proK treatment? Can the EZ1 robots purify as well as extract?
- How do the various EZ1 protocols differ? Which EZ1 protocol is the default protocol for evidence samples in our laboratory? Which protocol is used for known buccal swab samples?
- What is the optimal digest time for the extraction of evidence and reference samples (all extraction methods: EZ1s, Hamilton, Diffs)?
- How often should EZ1 piercing units be cleaned?
- When should the UV light decontamination feature be used on the EZ1s?
- Describe the quality control process for the extraction reagents and the investigator cartridges.
- What is the purpose of the Proteinase K in this procedure? DTT? Carrier RNA?
- How does a differential extraction work?
- Why is DTT added to the sperm extraction phase of a differential extraction?
- Why is it important to get a “clean” separation of the epithelial cells and sperm cells?
- Explain what the QIAcube does to assist in the separation during a differential extraction. Explain what happens during a run on the instrument.
- Why do we process the epithelial fraction of a differential extraction if we are looking for unknown male DNA? Why not just the sperm fraction?
- What procedures do you use to minimize the occurrence of contamination?

- When does contamination warrant re-extraction?
- Discuss the use of reagent blanks? What can they tell you? How does that differ from a negative control?
- Should bleach be used to clean the QIAcubes, EZ1s, and/or the Hamilton's during weekly and monthly maintenance? Explain why or why not.
- How are the buffers in the EZ1 DNA Investigator kit stored? The Prepfiler kit?
- Describe how magnetic beads function? Why are the wash steps important?
- Give an overview of the Prepfiler and Lyse & Prep chemistries.
- Why is air displacement pipetting important for the Hamilton STAR and STARlet?

9.16 Readings

9.16.3 See Required Reading Log – Module 8: DNA Extraction

9.17 Lectures

9.17.3 DNA Extraction – EZ1/QIAcubes (Tip Dance, Large Volume, and Trace Protocols)
9.17.4 DNA Extraction – Hamilton Starlet (Prepfiler Protocol)
9.17.5 DNA Extraction – Hamilton STAR (Lyse & Prep Protocol)

9.18 Practice

9.18.3 Demonstration by trainer and/or supervised performance
- 9.18.3.1 Trainer will demonstrate each of the extraction methods being introduced.
- 9.18.3.2 Trainer will discuss proper documentation and QC of extracted samples.

9.18.4 Practice Sets – Laboratory Skills

The amount and type of training samples should be appropriate given a particular procedure and may include the following:

- bloodstains
- saliva swabs
- hair specimens
- mixed stains (sperm/epithelial cell)
- dilution series

9.18.4.1 Three to five practice extraction sets will be performed per extraction method prior to administering the competency test.

9.18.4.2 These extractions can be used for quantification exercises in Module 9.

9.19 Conclusion

9.19.3 Competency Test
- 9.19.3.1 Practical
  - 9.19.3.1.1 A practical will be administered for each extraction method that is introduced.
  - 9.19.3.1.2 To be deemed competent in each protocol, each protocol must be performed as part of the practical (the same practical samples may be used for each protocol).

9.19.3.2 Written Exam

9.19.3.2.1 A written test will be administered.

9.19.3.2.2 A hardcopy of the exam will be stored within the training binder.

9.19.4 The trainee will be authorized to perform independent casework via a signed authorization memo.

9.19.5 The newly qualified analyst may be signed off to perform casework extraction, quantification, amplification, and capillary electrophoresis at separate times.

9.19.6 Oral Exam/Mock Trial

9.19.6.1 Successful completion of an oral exam is required.

9.19.6.2 Since there are several extraction methods utilized in the FBIO section, only one mock trial incorporating extraction is required.

10 Module 9: DNA Quantification

10.9 Duration

10.9.3 Approximately six weeks

10.10 Purpose

10.10.3 Enable trainee to quantify the amount of DNA present in extracted specimens.
10.10.4 To learn to make quantification standards, set up samples for quantification, and assess standard curves.
10.10.5 To learn to use real-time PCR results to gauge sample quality and achieve successful amplification.
10.10.6 To learn to set up plates, edit plates, and analyze quantity and quality of samples using the ABI Prism 7500 instruments and the HID Real-Time PCR Analysis Software.

10.11 Prerequisite

10.11.3 Module 2: Contamination and Evidence Handling
10.11.4 Module 7: Introduction to DNA and DNA History
10.11.5 May be done simultaneously with Module 8: DNA Extraction, Module 10: DNA Amplification, and Module 11: Capillary Electrophoresis

10.12 Theoretical Objectives

10.12.3 Trainee will be able to:

- Understand the necessity for DNA quantification of extracted samples prior to amplification.
- Describe three quantification methods for DNA and the advantages and limitations of each.
- Understand and explain the basis for DNA quantification utilizing the Quantifiler™ Trio kit.
- Understand the quality control measures associated with the DNA quantification method utilized in the laboratory.
- Understand the ability of the TECAN-150 EVOlution to setup DNA quantification.

10.13 Practical Skills

10.13.3 Trainee will be able to:

- Prepare dilutions of the human DNA standards.
- Use and maintain the ABI 7500.
- Perform the quantification procedure manually on extracted DNA samples.
- Perform the quantification procedure via TECAN on extracted DNA samples.
- Calculate the amount of DNA, total human and male.
- Complete the relevant documentation and quality control.
- Appropriately complete reprocessing paperwork.

10.14 Module Outline

I. Benefits of DNA quantification
- Preservation of sample for replicate analysis
- Distinguish between inhibition and insufficient DNA quantity
- PCR systems optimized for limited range of DNA quantity

II. Methods of DNA Quantification

A. Historical methods of quantification
- UV Spectroscopy
- Yield Gel
- Quantiblot™

B. qPCR Technology
- more accurate
- sensitivity
- primate specific
- can detect PCR inhibitors

III. Quantification Protocol
- Making the import sheet
- Loading the plate
  - Master mix and components
  - Samples
- Known DNA standards and the standard curve
- Documentation

IV. ABI 7500
- Overview
- Components
  - Computer and software
  - Halogen bulb
- Operating instructions
- Troubleshooting
- Cleaning, decontamination and maintenance

V. TECAN-150 EVOlution
- Overview
- Components
- Operating instructions
- Troubleshooting
- Cleaning, decontamination and maintenance

10.15 Theory

10.15.3 Once DNA has been extracted from a reference sample or evidentiary sample it is important to determine how much DNA is present before continuing with the amplification and typing

process. The PCR STR systems utilized in the laboratory are designed to work optimally using a specific range of input DNA. Quantification of DNA samples prior to amplification can insure that sufficient DNA is added to the PCR reaction and will aid in troubleshooting if no PCR product is obtained. In addition, using minimal volumes of DNA extracts will maximize the number of genetic marker tests or repeat analyses that can be performed. Additionally, it is a Quality Assurance Standard that all human DNA forensic samples be quantified prior to nuclear DNA amplification.

10.15.4 DNA from primate species may give signals similar to those obtained from equivalent amounts of human DNA.

10.15.5 Sample is consumed during analysis and may not be used again if the run fails. Does not detect DNA mixtures of same-gender contributors.

10.15.6 Assessment Questions

- Why is it important to quantitate the amount of extracted DNA in a sample prior to amplification?
- Can the quantification step be skipped in casework?
- Explain the quantification process with the ABI Prism 7500.
- What is the purpose of the IPC? What does it consist of?
- What is the range of Ct values for the IPS that indicates successful amplification? Large Target? Small Target? Male Target?
- What is an acceptable R^2 value for standard curves?
- What do the values for slope, y-intercept, and R^2 indicate and what happens if they are out of range?
- What is your conclusion if the large target shows a high Ct value and the IPC does not amplify?
- What is the purpose of the minor groove binder (MGB)?
- How should Quant Trio kits be stored?
- How are standard curve dilutions prepared? What quantification data should be evaluated after the first run of a new curve to assess whether the dilutions were successfully made?
- Which of the three quantification values, Large, Small, Male, will be used for amplification input volume calculations?
- How can the presence of an inhibitor be shown with Quant Trio?
- In general, should SAK samples move forward to amplification if no male DNA was detected? Are there exceptions? If so, what are they?
- When does contamination warrant re-amplification?
- What is a method for determining if the bulb in the 7500 needs to be replaced?

10.16 Readings

10.16.3 See Required Reading Log – Module 9: DNA Quantification

10.17 Practice

10.9.1 Demonstration by trainer and/or supervised performance

10.9.1.1 Trainee will observe trainer setting up and running a Quantifiler™ Trio run. Trainer will demonstrate the software for the 7500 and TECAN. Trainer will discuss proper documentation on worksheets, interpretation of standard curve, and the evaluation of results.

10.9.2 Practice Sets – Laboratory Skills

10.9.2.1 Trainee will set up and run a Quantifiler™ Trio run both manually and using the TECAN. The previously extracted samples from Module 8 may be used for the practice runs. Trainee will complete proper documentation of worksheets, interpretation of standard curves, and the evaluation of results.

10.9.2.2 Three to five practice sets will be performed prior to administering the competency test. This includes a minimum of three robotic set-ups and two manual set-ups.

10.18 Conclusion

10.18.3 Competency test

10.18.3.1 A practical test will be administered and performed in each method being introduced (manual and robotic).

10.18.4 Written test

10.18.4.1 A written test will be administered.

10.18.4.2 A hardcopy of the exam will be stored within the training binder.

10.18.5 The trainee will be authorized to perform independent casework via a signed authorization memo.

10.18.6 The newly qualified analyst may be signed off to perform casework extraction, quantification, amplification, and capillary electrophoresis at separate times

11 Module 10: DNA Amplification

11.1 Duration

11.1.1 Approximately six weeks

11.2 Purpose

11.2.1 Educate trainee on amplification of human DNA using the laboratory's on-line autosomal amplification kits.
11.2.2 Educate trainee on amplification of male DNA using the laboratory's on-line Y-STR amplification kits, if applicable.
11.2.3 To understand the theory behind each step in the amplification process.
11.2.4 To learn the purpose of each reagent in the amplification kits.
11.2.5 To become familiar with the use and purpose of positive and negative controls used in the procedure.

11.3 Prerequisite

11.3.1 Module 2: Evidence Handling
11.3.2 Module 7: Introduction to DNA and DNA History
11.3.3 May be completed simultaneously with Module 8: DNA Extraction, Modules 9: DNA Quantification, and Module 11: Capillary Electrophoresis.

11.4 Theoretical Objectives

11.4.1 Trainee will be able to:

- Explain who invented PCR and how the inventor was recognized.
- Describe the steps involved in amplification.
- Outline the advantages and disadvantages of PCR-based testing.
- Describe the different components and their purposes in an amplification mix.
- Describe the appropriate controls used and their purposes in the amplification of DNA samples
- Define and describe short tandem repeat (STR) markers.
- Define multiplexing and outline the STR markers contained in the laboratory's on-line autosomal. (And the Y-STR kit, if applicable)
- Perform equipment quality control on the thermal cyclers.
- Understand the ability of the TECAN-150 EVOlution to setup DNA amplification.

11.5 Practical Skills

11.5.1 Trainee will be able to:

- Determine the amount of template DNA necessary for amplification and prepare the appropriate dilutions of samples.
- Prepare the master mix and perform the amplification set-up of samples manually and robotically.

- Complete the amplification worksheet and appropriate documentation for quality control.
- Perform the critical reagent quality control on the laboratory's on-line autosomal kits. (And Y-STR kit, if applicable)
- Appropriately complete reprocessing paperwork.

11.6 Module Outline

I. Advantages of PCR
- Amount of DNA template required
- DNA degradation
- Analysis time
- Multiplexing

II. Disadvantages and considerations of PCR
- Mixed samples
- Preferential amplification and stochastic effects
- Inhibition
- Contamination

III. Master mix ingredients
A. Purpose of each component
- DNA polymerase
- MgCl2
- Deoxynucleotide triphosphates
- primers
- bovine serum albumin (BSA)

B. Modification of stringency of amplification by changing concentrations of certain components in master mix

IV. Steps involved in amplification
- Purpose of each step
- Modification of stringency of amplification by changing times/temperatures of each step

V. Appropriate controls
- Thermal cycler quality control procedure

VI. TECAN-150 EVOlution
- Overview
- Components
- Operating instructions
- Troubleshooting
- Cleaning, decontamination and maintenance

VII. Background Information on STRs

VIII. General information on laboratory's on-line autosomal and Y-STR kits

11.7 Theory

11.7.1 The amplification and analysis of short tandem repeats (STRs) is at the forefront of forensic DNA technology. The use of DNA amplification by PCR (polymerase chain reaction) to make millions of copies of specific DNA sequences enables the analysis of small quantities of DNA or degraded DNA usually encountered in forensic samples. The use of multiplexed STRs allows for more rapid analysis time with less sample consumption.

11.7.2 Assessement Questions

- What are the three fundamental steps in PCR? Describe what occurs in each step. Include temperatures.
- What are the standard PCR reaction components and their functions?
- Per protocol, what is the optimal template target for amplification for Globalfiler? Why was this target chosen? What is the maximum input volume?
- What are the Globafiler loci and on which chromosomes are they located?
- What is a degenerate primer? Which loci in Globalfiler have them?
- What is a DNA polymerase?
- What is the plateau effect and how does it affect the DNA sample?
- What are some of the factors that inhibit PCR amplification and why? What steps can the analyst take to overcome inhibition problems?
- How does a 2-step PCR process different from a 3-step PCR process? What is Globalfiler?
- What is the purpose of the 60 degree hold following temperature cycling?
- What are the components of the Globalfiler kit?
- What are the amplification conditions for Globalfiler?
- What does $MgCl_2$ do?
- Who invented the PCR process and when?
- Compare and contrast PCR and RFLP. Why has forensic science embraced PCR over RFLP?
- What is the quality control procedure for the amplification kits? Where is this documentation found?
- What does UV light do to DNA?
- What is the error rate of Taq? Explain what this means and concerns with its use in forensic testing.
- Why are all PCR products approximately the same length?
- What would you expect to see if the annealing temperature was too low?
- What might happen if you increase primer concentration?
- What is “-A” and what is done to overcome this potential issue?
- What information needs to be verified on the re-amplification request?
- What information needs to be verified in the lab before you start re-amping your samples?
- What order should the sample types be in on the re-amplification request?

11.8 Readings

11.8.1 See Required Reading Log – Module 10: DNA Amplification

11.9 Practice

11.9.1 Demonstration by trainer and/or supervised performance

11.9.1.1 Trainer will demonstrate the proper technique for setting up the amplification reactions manually and robotically, if applicable.

11.9.1.2 Trainer will discuss proper documentation on worksheets.

11.9.2 Practice Sets – Laboratory Skills

11.9.2.1 Trainee will set up amplification reactions from previously extracted and quantified samples along with utilizing the proper quality control worksheets and controls manually and on the TECAN-150. The previously extracted and quantified samples from Modules 8 and 9 may be used for the practice runs.

11.9.2.2 Three to five practice autosomal amplifications will be performed prior to adminstering the autosomal competency test. This includes a minimum of three robotic set-ups and two manual set-ups.

11.10 Conclusion

11.10.1 Competency test

11.10.1.1 A practical test will be administered, and performed in each technology and each method being introduced (manual and robotic).

11.10.2 Written test

11.10.2.1 A written test will be administered.

11.10.2.2 A hardcopy of the exam will be stored within the training binder.

11.10.3 The trainee will be authorized to perform independent casework via a signed authorization memo.

11.10.4 The newly qualified analyst may be signed off to perform casework extraction, quantification, amplification, and capillary electrophoresis at separate times

12 Module 11: Capillary Electrophoresis

12.1 Duration

12.1.1 Approximately six weeks

12.2 Purpose

12.2.1 Educate trainee on the proper use of the ABI 3500 and software, and how to troubleshoot problems with the instrument and make recommendations on fixing any encountered problems.

12.3 Prerequisite

12.3.1 Module 2: Contamination Concerns
12.3.2 Module 7: Introduction to DNA and DNA History
12.3.3 May be done simultaneously with Module 8: DNA Extraction, Modules 9: DNA Quantification, and Module 10: DNA Amplification

12.4 Theoretical Objectives

12.4.1 Trainee will be able to:

- Understand how to utilize the ABI 3500 software.
- Describe the components used by the computer programs for analysis of data.
- Evaluate data quality from the ABI 3500.
- Care for and properly clean the instrument.
- Describe what steps to take in troubleshooting a problem with the instrument.
- Archive data and maintain the computers.
- Describe the ability of the TECAN-150 EVOlution to setup CE plates.

12.5 Practical Skills

12.5.1 Trainee will be able to:

- Perform capillary electrophoresis on previously amplified DNA product.
- Perform both manual and robotic set-up.
- Clean the instrument and demonstrate proper care of the instrument.
- Perform daily, weekly, monthly, annual maintenance and performance checks.
- Appropriately complete reprocessing paperwork.

12.6 Module Outline

I. Multicomponent Analysis
   A. Spectral
      - Purpose of the spectral

- Making a spectral

B. Modules
- Filter set J6
- POP-4 polymer
- Urea crystal formation

C. Off-scale data
- Relative Fluorescence Units (RFU)
- Causes

II. Proper care and cleaning of the instrument

A. Parts of the instrument
- Laser
- CCD
- Mother board
- Autosampler
- Syringe pump
- Heat block
- Pump block

B. Instrument break-down and cleaning
- Reasons
- Frequency
- Pump block
- Water/Buffer vials and septa
- Capillary storage

C. Rebooting the instrument
- Soft reboot
- Hard reboot
- Resetting the instrument

III. Sample preparation/electrophoresis

A. Instrument set-up
- Spatial alignment
- Loading the instrument with polymer
- Making buffer
- Plate records
- Injection lists
- Sample set-up
- Allelic Ladder
- Formamide/LIZ
- Adding sample

B. TECAN-100 EVOlution
- Overview
- Components

- Operating instructions
- Troubleshooting
- Cleaning, decontamination and maintenance

IV. 3500 Data Collection and Software
- Laser
- CCD camera
- Fluorescent dye labels
- Manual control of the instrument
- Wizards

IV. Maintenance of the 3500
- Capillary changes
- Polymer lot #
- Analysis parameters
- Spectral runs

IV. Case folder
- Electropherograms
- Controls
- Injection Lists
  - Reasons for re-injection

12.7 Theory

12.7.1 Short tandem repeat (STR) markers are polymorphic DNA loci that contain a nucleotide sequence that has been repeated. The loci can be amplified using fluorescent dye-labeled primers. The PCR products are then separated by size using capillary electrophoresis.

12.7.2 When problems arise during the processing of DNA samples, the analysts must be able to use their knowledge to systematically reason through the possible causes and solutions to these problems.

12.7.3 Assessment Questions

- Where in the 3500xL data collection software can the run parameters be found?
- How often should the polymer and buffers be changed? How often are water washes of the pump block and the water trap performed?
- What are the “default” injection times used on the 3500xL? Will these times ever change?
- Draw the electric circuit that is created when the electrophoretic process is underway.
- How is DNA loaded into each capillary? What voltage is used for injection? What voltage is used for electrophoresis?
- Can environmental temperature changes affect the run? If so, explain how.
- What does the abbreviation RFU stand for?

- What is the purpose of the formamide? Why is the formamide de-ionized?
- Why is the array filled with polymer at the beginning of a run?
- Why is it important for the array to remain in buffer after electrophoresis is completed?
- What is the dashboard?
- Does the array have an expiration date? Does it have a limit on the number of injections? Are either of these hard stops?
- Polymer, anode buffer, and cathode buffer may be left on the 3500xL for how many days?
- What are RFID Tags? What consumables/reagents are tracked by the instrument/data collection software using RFID tags? What information is recorded via RFID?
- What is the conditioning reagent?
- Does the 3500 capillary expire? Explain your answer.
- What is the spatial calibration and when should it be performed?
- What is a spectral? What reagents are required to make a new spectral and when should it be generated? What must be met before it is acceptable?
- How many borrowing events are allowed to still pass a spectral?
- How often should the cathode and anode buffers be changed?
- What is a ladder? What is its function?
- Which fluorescent dye is used to detect the internal lane standard?
- Explain fluorescent detection.
- The internal lane standard consists of how many peaks and what is the base pair size of each? What is the function of this standard?
- How is a sample loaded onto a column? What is this process called?
- How much amplicon is added or can be added to the formamide/LIZ mixture?
- What is the daily, weekly, monthly required instrument maintenance?

12.8 Readings

12.8.1 See Required Reading Log – Module 11: Capillary Electrophoresis

12.9 Practice

12.9.1 Demonstration by trainer and/or supervised performance

12.9.1.1 Trainer will demonstrate proper technique for cleaning and setting up the 3500, analyzing and interpreting data, and troubleshooting problems with the instrument. Plate set-up may be both manual and robotic.

12.9.2 Practice Sets – Laboratory Skills

12.9.2.1 Trainee will load previously amplified samples on the 3500. Trainee will troubleshoot problems using the data and suggest/implement ways to remedy these problems. Plate set-up should be both manual and robotic.

12.9.2.2 Three to five practice runs will be performed prior to administering the competency test. This includes a minimum of three robotic set-ups and two manual set-ups.

12.10 Conclusion

12.10.1 Competency test

12.10.1.1 A competency test will be administered by the trainer, and performed in each method being introduced (manual and robotic).

12.10.2 Written test

12.10.2.1 A written test will be administered by the trainer.

12.10.2.2 A hardcopy of the exam will be stored within the training binder.

12.10.3 Oral Exam/Mock Trial

12.10.3.1 Successful completion of an oral exam and mock trial is required.

12.10.4 The trainee will be authorized to perform independent casework via a signed authorization memo.

12.10.5 The newly qualified analyst may be signed off to perform casework extraction, quantification, amplification, and capillary electrophoresis at separate times.

13 Module 12: DNA Interpretation

13.1 Duration

13.1.1 6 weeks

13.2 Purpose

13.2.1 To provide experience in interpreting DNA profiles, including how to analyze and interpret data from the instrument using GeneMapper ID-X.
13.2.2 This shall include mixtures of varying concentrations and in understanding the effects of DNA quantity on typing results.
13.2.3 To become familiar with the GeneMapper ID-X software.
13.2.4 To develop an understanding of how genotypes are assigned using in-lane standards and allelic ladders
13.2.5 To become familiar with how the stutter percentages were calculated by the laboratory.
13.2.6 To become familiar with the sensitivity of the system and to learn when re-amplification is necessary vs. re-injection.
13.2.7 To become familiar with the ladder, amplification control, and reagent blank review process.
13.2.8 To become familiar with biological samples containing DNA mixtures and deconvolution of two-person mixtures.
13.2.9 To become familiar with how to determine the minimum number of contributors to a STR profile.
13.2.10 To become familiar with the limitations of detecting minor components in a mixture.

13.3 Prerequisite

13.3.1 Approval by Biology Manager and/or Technical Leader
13.3.2 May be done simultaneously with Module 14: CODIS

13.4 Theoretical Objectives

13.4.1 Trainee will be able to:

- Interpret data from the 3500xl
- Describe the challenges and limitations involved when working with low level DNA samples.
- Describe how DNA quantity of a sample affects typing results.
- Describe the criteria for determining if a sample is single source or a mixture.
- Interpret mixtures, including distinguishing major/minor components and deducing from intimate samples.

13.5 Practical Skills

13.5.1 Trainee will be able to:

- Analyze and interpret dilution and mixture samples using the 3500 and corresponding software.
- Examine electropherograms to differentiate between the major and minor components, stutter, and artifacts.
- Conclude which individuals would be excluded or included in mixed samples.
- Explain stochastic effect, stochastic threshold, and analytical threshold.

13.6 Module Outline

I. GeneMapper ID
- Analysis parameters
  - Setting threshold values
  - Light smoothing
  - Analysis range and excluding primer peak
  - Local southern method for sizing
- Examination of Raw Data
- Allelic Ladder
- Internal Lane Standard
  - Defining the internal lane size standard
  - Examining the floating base pair fragment
- Sizing of peaks
  - Offsets
  - Virtual alleles
  - Labeling peaks with base pair sizing, peak height, etc.
- Sizing of peaks
  - Off-ladder alleles, spikes, pull-up
  - Stutter
  - Minus A
- Microvariants
  - Sizing

II. Evaluation of data
- Controls
  - Positive control
  - Negative control
  - Reagent blanks
  - Allelic ladder

III. Troubleshooting
- Identifying problems with data
- Determining causes of problems
  - Urea crystals
  - Inhibition
  - Formation of formate ions

- Bubbles
- Breakdown of buffer vial septa
- Old buffer
- Bad injections
- Bad laser
- Bad CCD camera
- Dirty capillary window or bad capillary
- Arcing
- Temperature fluctuations

- Determining solutions to problems

IV. Interpretation of analyzed data

- Single source
- Second reading by qualified reviewer
- Stochastic effect and allelic dropout
- Degraded DNA
- Low template number DNA
- Dilution Studies
  - DNA concentrations requirements
  - Sensitivity within each genetic analyzer
  - Balance across loci
  - Heterozygous peak height balance
- Mixtures
  - Peak height ratios
  - Major component
  - Minor component
  - Two-person mixtures
  - Three plus person mixtures
- Mixture Studies
  - Major and minor component identification
  - Balance across loci
  - Heterozygous peak height balance
  - Minor peak vs. stutter product vs. artifact
  - Diploid vs. haploid cell contributions
  - Deductions
- Comparisons
  - Inclusions
  - Exclusions

13.7 Theory

13.7.1 Data gathered by the instrument must be interpreted by qualified analysts. The conclusions drawn from the data are used to identify inclusions and/or exclusions. This

enables the analyst to determine if a DNA profile could or could not have originated from an individual.

13.7.2 Many evidentiary items contain minimal stains with limited quantities of DNA. It is important to understand how the quantity of DNA in a sample can affect the typing results.

13.7.3 Mixtures of DNA from two or more individuals are regularly encountered. Experience in interpreting these mixtures and attempting to identify possible contributors is critical for inclusion/exclusion purposes as well as for CODIS entry.

13.7.4 Assessment Questions

- What are some criteria for identifying a sample as a mixture? Explain the theory behind each criterion.
- What is a stutter peak? What Globalfiler locus shows the greatest stutter percentage?
- What is stochastic drop out? What is the stochastic threshold for this laboratory? How was it derived? How might drop out affect your interpretation?
- What are the Globalfiler loci and on which chromosomes are they located?
- Define analytical threshold? What is the analytical threshold for this laboratory? How was it derived?
- Are amelogenin alleles STRs? Explain.
- What does the abbreviation RFU stand for?
- What is the LIZ internal lane standard used for? What information about the STR peaks is obtained by running the LIZ standard with each sample? How many LIZ peaks are necessary for analysis? What are their base pair sizes?
- What are allelic ladders used for? How should they be assessed? How does the ladder work in conjunction with the LIZ standard to assign STR alleles?
- What causes off-scale data? What are your options to overcome it?
- Discuss how to assess the raw data to determine whether a peak is genuine or an artifact. What artifacts can be edited on the electropherograms and how is this done?
- What would you do if you discovered contamination in a blank control sample?
- What is a degenerate primer? Which loci in Globalfiler have them and how might this affect a comparison between legacy data and a Globalfiler profile?
- Describe the cause of the following data issues and how they can be confirmed and/or overcome.
    - No Sizing
    - Off-scale data
    - Pull up
    - Raised baseline
    - Signal spikes
    - Split peaks/shoulders

- What is a microvariant? What additional analytical steps are necessary if a microvariant, labeled as "OL" by the software, is detected? What documentation is required in the case file?
- If a true allelic peak is labeled "OMR", how would you associate it with a locus? Is reinjection of this sample required for confirmation?
- How would you investigate the following anomalous results?
    - Apparent cross-contamination from evidence to a reagent blank
    - Analyst profile in an evidentiary sample
    - Apparent cross contamination from evidence to a negative PCR control
    - Analyst profile in a reagent blank
    - No positive control alleles detected, all other samples appear okay
    - Off scale LIZ
- Why is it important to get a 'clean' separation of the epithelial cells and sperm cells?
- How can degradation be distinguished from inhibition?
- What is preferential amplification and why does this occur? Is this a problem when analyzing samples using STR technology? Why or why not?
- If an inhibitor is suspected, should more or less extract be amplified? Explain.
- When is a profile considered a mixture?
- How do you determine the minimum number of contributors to an STR profile? Does stutter play a role? How can peaks below the analytical threshold affect your interpretation?
- What precautions are used to ensure that allelic drop-out has not occurred?
- Why do we process the epithelial fraction of a differential extraction if we are looking for unknown male DNA? Why not just the sperm fraction?
- What is the ratio in determining major/minor mixtures?
- What is the role of minimum peak height ratio expectations in a two-person mixture interpretation?
- Can peaks below stochastic threshold be used for comparison purposes?
- When is a two-person mixture considered inconclusive? A mixture of three or more contributors?

<u>Y-STRs</u>

- Discuss the difference between a haplotype and a haplogroup. How would haplogroup information be used to determine a person's ancestry?
- Discuss the mutation rates of Y chromosome loci and how this affects the diversity of Y haplotypes.
- Discuss multi-copy loci. What is the difference between multi-copy locus and a duplication?
- How are the loci on the Y chromosome inherited? How does the affect interpretation of a match?
- Is there any region on the Y chromosome that exhibits recombination?
- Discuss the duplicated loci present in the Yfiler Plus kit and their nomenclature.
- What loci comprise the European Minimal Haplotype? The SWGDAM recommended core set?

- List the loci and their dye labels for the YF+ amplification kit.
- Are all the loci in the YF+ kit compromised of tetranucleotide repeats? If not, what are the differences?

13.8 Readings

13.8.1 See Required Reading Log – Module 12: DNA Interpretation

13.9 Practice

13.9.1 Single Source and Dilution Exercise

13.9.1.1 Analysis of samples previously prepared is acceptable, including validation data. The analyst may not need to prepare separate samples. If needed, prepare samples following the directions below:

13.9.1.1.1 Obtain a DNA extract previously quantitated as 1.00 ng/ul. Prepare a series of serial dilutions from this extract to result in samples of 2 ng, 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng, 0.0312 ng, and 0.0156 ng.

13.9.1.1.2 Amplify the samples as outlined in Module 10: DNA Amplification.

13.9.1.1.3 Analyze the samples as outlined in Module 11: Capillary Electrophoresis

13.9.1.2 One Data Set Exercise will be introduced to provide familiarity with analyzing DNA samples, identifying analytical and stochastic thresholds, identifying various artifacts routinely encountered, and interpreting full and partial profiles of single source samples.

13.9.1.3 Results will be reviewed by the trainer and/or Technical Leader and the trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

13.9.2 Mixture Exercises and Quiz

13.9.2.1 Analysis of samples previously prepared is acceptable, including validation data. The analyst may not need to prepare separate samples. If needed, prepare samples following the directions below:

13.9.2.1.1 Obtain two DNA extracts previously quantitated as 1.00 ng/ul - one from a male (donor A) and one from a female (donor B).

13.9.2.1.2 Prepare mixtures as follows with total amount of genomic input DNA mixed at each ratio as 1.00 ng:

- 1:1 ratio of A:B
- 3:1 ratio of A:B
- 5:1 ratio of A:B
- 10:1 ratio of A:B
- 20:1 ratio of A:B
- 3:1 ratio of B:A
- 5:1 ratio of B:A
- 10:1 ratio of B:A
- 20:1 ratio of B:A

13.9.2.2 Amplify the samples as outlined in Module 10: DNA Amplification.

13.9.2.2.1 Analyze the samples as outlined in Module 11: Capillary Electrophoresis.

13.9.2.3 Two Data Set Exercises will be introduced to provide familiarity with two-person and three (or more) person mixtures and their deconvolution.

13.9.2.4 A Mixture quiz

13.9.2.4.1 A mixture quiz will assess the trainee's understanding of deconvolution of two and three (or more) person mixtures.

13.9.2.4.2 The quiz will be reviewed by the trainer and/or Technical Leader and the trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

13.9.3 Comparison Exercises and Quiz

13.9.3.1 One Data Set Exercise will be introduced to provide familiarity with the process of comparing reference samples to evidentiary samples and determining which individuals would be excluded or included.

13.9.3.2 Inclusion/Exclusion Quiz

13.9.3.2.1 An Inclusion/Exclusion Quiz (consisting of a minimum of five sets of DNA mixtures) will assess the trainee's understanding of comparing reference samples to evidentiary samples.

13.9.3.2.2 The quiz will be reviewed by the trainer and/or Technical Leader and the trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

13.9.4 All completed documentation from these exercises and quizzes will be included and retained in the trainee's competency notebook.

Subsection of Module 12: Probabilistic Genotyping

13.10 Duration

13.10.1 Approximately six to eight weeks

13.11 Purpose

13.11.1 To familiarize the trainee with using the probabilistic genotyping system STRmix™ as a tool to assist in the interpretation of DNA typing results.

13.12 Prerequisite

13.12.1 Module 12: DNA Interpretation
13.12.2 May be done simultaneously with Module 14: CODIS

13.13 Theoretical Objectives

13.13.1 Trainee will be able to:

- Describe the basic principles of probabilistic genotyping and STRmix™ including likelihood ratios, biological modeling, mass parameters, Markov Chain Monte Carlo (MCMC), and Metropolis Hastings.
- Utilize STRmix™ to interpret DNA profiles, conduct mixture deconvolution, and compare associated reference samples.

13.14 Practical Skills

13.14.1 Trainee will be able to:

- Propose propositions to best explain the evidence and assign a number of contributors to profiles.
- Evaluate data and statistical results in the form of a likelihood ratio calculated by STRmix™.

13.15 Module Outline

I. Overview of Probabilistic Genotyping
- Comparing Interpretation strategies
  - CPI v RMP v LR
  - Thresholds
  - Deconvolution
  - Number of contributors
  - NRC II recommendation 4.1 and 4.2
- Science behind STRmix™
  - Biological Models and TAP
  - MCMC
  - Metropolis Hastings
  - Number of Contributors
- STRmix™ Diagnostics
  - Saturation
  - Gelman Rubin
  - Effective Sample Size
  - Log (likelihood)
  - Variance
- Use of STRmix™by the HFSC FB section
  - Software
- Population databases

13.16 Theory

13.16.1 Prior to STRmix™, profile interpretation was determined by practitioner experience, rules, and guidelines for DNA profile behavior. The STRmix™ program refines this process of developing weights by allowing each genotype set to be given a weight of any value on a continuous spectrum between 0 and 1. STRmix™ will use the data gathered by the 3500 instrument and input from qualified analysts to interpret and deconvolute DNA profiles. STRmix™ will also be used to compare reference DNA profiles to casework profiles to generate a measure of weight of the evidence (likelihood ratio) in relation to a pair of propositions. The results generated from the STRmix™ software will be evaluated by a qualified analyst.

13.16.2 Assessment Questions

For all questions and exercises in this handout, you may use any resources available to you. Responses should be thorough and technical in nature. You will have the opportunity to answer similar questions as if to a jury in your mock trial/oral exam.

Define the following terms:

1. STRmix
2. Probabilistic Genotyping
3. MCMC
4. DART
5. Biological models
6. Mass parameters
7. LR
8. Hypothesis/proposition
9. Metropolis Hastings
10. HPD
11. 99% 1-sided confidence interval
12. Stratified statistic
13. Unified statistic
14. Relatedness calculation
15. ESS
16. Gelman Rubin
17. “N!”
18. NRC II recommendation 4.2
19. NRC II recommendation 4.1
20. SEED
21. LSAE
22. Degradation
23. LUS
24. Iteration
25. Allele Variance
26. Stutter Variance
27. Log(likelihood)

28. "Q"
29. Weight
30. "F"
31. Component ≥ 99%
32. FST
33. NOC
34. Mixture proportion

Answer the following questions:

35. Which biological models are utilized by STRmix? How?
36. What does an LR 0.002 indicate?
37. What does an LR 1.0 indicate?
38. What does an LR 1,000,000 indicate?
39. What is your saturation limit for STRmix interpretation?
40. Does STRmix account for drop-in? If so, how?
41. How do you choose the two hypotheses when setting up your propositions?
42. What impact does conditioning, if any, have on the LR?
43. Was MCMC developed for STRmix™?
44. How does STRmix™ determine the NOC for a given mixture?
45. Does STRmix™ account for sampling variation? If so, how?
46. How were the HFSC STRmix™ parameters determined?
47. After referring to sections B, G, and K of the internal validation, provide 3 causes for a false exclusionary LR.
48. After referring to sections B, G, and K of the internal validation, provide 3 reasons why STRmix™ may fail to interpret a data set.
49. Generally speaking, what effect does under-estimating the number of contributors have?
50. Generally speaking, what effect does over-estimating the number of contributors have?
51. Can you ever truly know the number of contributors to an evidentiary mixture?
52. Which diagnostics are used to evaluate a STRmix™ interpretation? For each diagnostic provided, what is the expected value and how do you determine if there may be a problem?

13.17 Readings

13.17.1 See Required Reading Log – Module 12: Probabilistic Genotyping

13.18 Practice

13.18.1 Training exercises

13.18.1.1 Three Data Set Exercises will be introduced to provide familiarity with inputting data into the STRmix™ software and evaluating the results generated.

13.18.1.2 One Proposition Exercise will be introduced to provide familiarity with formulating propositions for input into the STRmix™ software.

13.18.1.3 These exercises will be reviewed by the trainer and/or Technical Leader and the trainee will repeat any items not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

13.19 Competency Set

13.19.1.1 A practical set will be administered to assess the trainee's understanding and application of the STRmix™ software.

13.19.1.2 The set will be reviewed by the trainer and/or Technical Leader. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

13.19.2 Oral Exam/Mock Trial

13.19.2.1 Successful completion of an oral exam and mock trial encompassing DNA interpretation, DNA statistics, and DNA report writing is required.

14 Module 13: DNA Statistics

14.1 Duration

14.1.1 three weeks

14.2 Purpose

14.2.1 To familiarize the trainee with the theory of population statistics and its application to the evaluation of DNA evidence.

14.3 Prerequisite

14.3.1 Module 12: DNA Interpretation
14.3.2 May be done simultaneously with Module 14: CODIS

14.4 Theoretical Objectives

14.4.1. Trainee will be able to:

- Describe the basic principles of population statistics including Hardy-Weinberg equilibrium, Linkage equilibrium, and population substructure, the ceiling principle, and random match vs. likelihood ratios.
- Utilize the statistical programs and databases used by the Houston Forensic Science Center Forensic Biology section.

14.5 Practical Skills

14.5.1 Trainee will be able to:

- Manually calculate random match probability (RMP) and combined probability of inclusion (CPI) using the NRC II Recommendation 4.1 formulae and the Population frequency tables.
- Apply the Popstats software from the FBI

14.6 Module Outline

I. Overview of population genetics
- PCR-based systems
- Randomly Mating Populations
  - Hardy-Weinberg (HG) equilibrium
  - Linkage equilibrium (LE)
- Population structure
- Subpopulations
- Statistical considerations
  - The reference database
  - Match Probability (random match) vs. Likelihood Ratio (LR)

- Bayes' Theorem
- Identification of suspect by database
- Uniqueness

- DNA in the courts

II. The 1992 NRC Report
- The validity of DNA typing
- The use of DNA for exclusion
- Changes since the 1992 report
- The Ceiling Principles
- Paternity testing

III. Conclusions and Recommendations of the NRC

IV. Use of Statistics by the HFSC FB section
- Significance estimation
  - RMP
  - CPI
  - Minimum and Null Allele frequencies
  - Off-ladder alleles
  - Software
- Population databases
- Population frequency tables

14.7 Theory

14.7.1 Once a match has been identified and an individual included as a possible source of evidentiary material, the significance of that match is estimated to allow investigators, the legal sector, and ultimately a jury of lay persons, to place the appropriate emphasis on the conclusion. Significance estimation will be expressed as an inverse probability of inclusion and likelihood ratios will not be calculated. The latest version of the FBI's Popstats software will be configured and installed to calculate single source and mixed source significance estimates.

14.7.2 Assessment Questions

- When is a random match (RMP) calculation appropriate? Combined probability of inclusion (CPI)?
- When is $2p\text{-}p^2$ used? 2pq?
- Why are loci with alleles below stochastic threshold not used in CPI calculations? Is it ever acceptable to use alleles below the stochastic range to calculate a frequency estimate?
- What is the mathematical relationship between the Probability of Inclusion (P_I) and the Probability of Exclusion (P_E)?
- What is the theta correction factor? Why is it used? What levels are recommended for use in different situations?

- Discuss the minimum allele frequency: What is it? How is it calculated? When is it used in frequency estimate calculations?
- Why is it mathematically acceptable to multiply the frequency estimates obtained for each individual locus together to determine the frequency for the entire profile?
- Why are mixture profiles that have no loci available for stats deemed "insufficient for comparison" even if there are alleles above the stochastic threshold? Are there any conclusions that can be drawn from these mixtures?
- How should statistics be applied to forensic mixtures with allelic activity under the threshold?
- If you have a single source profile with a heterozygotic locus but both alleles are below the stochastic threshold (and above analytical), can it be used for stats?
- In 2015, the FBI issued an erratum to the original allele frequencies published in 1999 and 2001. What were the issues that caused the errors in the original allele frequencies? What is the effect of the discrepancies on the profile statistics?
- How does linkage equilibrium affect STR analysis?
- What does independence mean as related to population genetics?
- Is the database HFSC uses in Hardy-Weinberg equilibrium?
- How is the product rule applied?
- How do allele frequencies differ from genotype frequencies?
- How can the term "observational bias'" be applied towards DNA analysis? How can this be avoided?
- What is an obligate allele in terms of RMP? When is it used?

<u>Paternity</u>

- What is an obligate allele with regards to paternity analysis?
- How many exclusions are required for paternity? Why?
- What is "reverse parentage"? How is this different from a traditional paternal trio?

<u>Y-STRs</u>

- (YSTRs) Describe the differences between the random match probability and the counting method. Describing the counting method as you would to a jury.
- (YSTRs) What interpretations can be performed on a mixed YSTR profile?
- (YSTRs) Can the product rule be used to determine the rarity of the loci on the Y chromosome? Why or why not?

14.8 Readings

14.8.1 See Required Reading Log – Module 13: DNA Statistics

14.9 Practice

14.9.1 Training exercises

14.9.1.1 Two Data Set Exercises will be introduced to provide familiarity with calculating statistics for single source profiles and two-person or three (or more) person mixtures.

14.9.1.2 The trainee will manually calculate a single source significance estimation and a mixed source significance estimation using the NRC II Recommendation 4.1 formulae and the Population frequency tables.

14.9.2 Statistics Written Exam

14.9.2.1 A statistics exam will assess the trainee's understanding and application of calculating single source, and two or three (or more) person mixtures.

14.9.2.2 The exam will be reviewed by the trainer and/or Technical Leader. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer and/or Technical Leader.

15 Module 14: COmbined DNA Index System (CODIS)

15.1 Duration

15.1.1 One to two days

15.2 Purpose

15.2.1 To inform and instruct the trainee with the uses and laws related to the COmbined DNA Index System.

15.3 Prerequisite

15.3.1 May be done simultaneously with Modules 12: DNA Interpretation and Module 13: DNA Statistics

15.4 Theoretical Objectives

15.4.1 Trainee will be able to:

- Discuss and define what a database is
- Distinguish between different databases (NDIS, SDIS, LDIS)
- Distinguish between different indexes (Forensic Unknown, Forensic Partial, Legal, etc).
- Describe the background and state and federal laws associated with the collection of samples for CODIS.

15.5 Practical Skills

15.5.1 Trainee will be able to:

- Determine which profiles should be entered into the CODIS database.

15.6 Module Outline

I. History
   - DNA Identification Act-1994
   - House Bill 40, Texas 74th Legislature-Collection of convicted Sex Offender Database.
   - House Bill 1188, Texas 76th Legislature-Expands collection to murder, aggravated assault, burglary of habitation, offense or conviction of which registration as a sex offender is required.

II. NDIS-National DNA Index System

III. SDIS-State DNA Index System

IV. LDIS-Local DNA Index System

V. Specimen Category/Identification
   - Forensic Unknowns
   - Suspect Knowns
   - Forensic Mixtures-resolving mixtures for CODIS entry

- Peak height ratio calculations
- Major/minor component

VI. CODIS reporting

VII. State CODIS Laboratory

- Forensic Hits
  - Case to case
  - Case to suspect
  - Offender Hits
  - Autosearching
  - Uploads
  - Backups

15.7 Theory

15.7.1 "The CODIS blends forensic science and computer technology into an effective tool for solving violent crimes. CODIS enables Federal, State and Local crime laboratories to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders." The FBI's DNA and Databasing initiatives, US Department of Justice

15.7.2 Assessment Questions

- What does CODIS stand for? NDIS? SDIS? LDIS?
- Name the CODIS Administrator, the alternate, and their functions?
- What CODIS Programs are routinely used for casework?
- What security measures are taken to limit access to the CODIS data, software, and server?
- What factors are taken into consideration when determining whether a single source profile is suitable for database entry or searching? Discuss with respect to CODIS category, eligibility, profile completeness, and partial or low-level profiles.
- What are the differences between the Forensic Unknown and Forensic Partial Indexes? Discuss with respect to profile completeness, search parameters, and SDIS vs NDIS?
- What is the partial locus indicator? When should it be marked? What affect does it have on downstream match assessments?
- What additional factors should be considered if the profile is a mixture?
- What does using the "+" symbol when entering allele calls accomplish? When would this be a useful tool? What are potential drawbacks of assigning an allele with "+"?
- What are the differences between high, moderate, and low stringency searching?
- How many loci are required for acceptance of an evidence profile at NDIS? Are there loci that are not "counted" when trying to determine number of loci for upload to NDIS? What other thresholds must be met?
- Discuss the use of the Match Estimator tool: When in the CODIS process should it be used and for what types of profiles? What affect does the frequency have upon specimen category?

- Can a microvariant allele be entered into Match Estimator? What should be entered instead and why? Are there times when an allelic "placeholder" is necessary even when a called allele is not a microvariant?
- What formula is used to calculate the frequency of a single allele entry in Match Estimator?
- Can mixtures be entered in CODIS?
- What is familial searching?
- Can YSTRs be entered into CODIS?

15.8 Readings

15.8.1 See Required Reading Log – Module 14: COmbined DNA Index System

15.9 Practice

15.9.1 CODIS Deduction

15.9.1.1 One Data Set Exercise will be introduced to provide familiarity with profile deduction for CODIS entry.

15.9.2 Eligibility Determination

15.9.2.1 One Data Set Exercise will be introduced to provide familiarity with determining CODIS eligibility of forensic profiles based upon case scenario.

15.10 Conclusion

15.10.1 Fingerprinting

15.10.1.1 Trainee will coordinate with the CODIS administrator to be fingerprinted as part of the application for access into the CODIS system.

15.10.2 Complete Online CODIS Webinar and Exam

15.10.2.1 Trainee will complete the NDIS online webinar followed immediately by assessment questions to allow access into the CODIS system.

16 Module 15: DNA Report Writing

16.1 Duration

16.1.1 Approximately three weeks

16.2 Purpose

16.2.1 To provide guidelines for DNA casework report writing and for technical reviews and administrative case reviews.
16.2.2 To become skilled at expressing DNA STR typing results in a clear, concise, and technically correct manner.
16.2.3 To become familiar with the legal aspects of DNA typing, including how to present aspects of scientific testimony on DNA evidence to a lay audience.
16.2.4 To become familiar with issues relating to DNA technology and forensic DNA testing frequently addressed in court.

16.3 Prerequisite

16.3.1 Modules 12: DNA Interpretation
16.3.2 Module 13: DNA Statistics
16.3.3 Module 14: CODIS

16.4 Theoretical Objectives

16.4.1 Trainee will be able to:
- Write clear, concise reports that contain DNA analysis.

16.5 Practical Skills

16.5.1 Trainee will be able to:
- Compile DNA results into a concise conclusion utilizing the LIMS.
- Produce a report in an easily understandable format.

16.6 Module Outline

I. General Information
   - Previous laboratory reports
II. Requested Analysis
   - Laboratory's canned statements
   - Special requests made by the officer
III. Results of DNA analysis
IV. Statistics
V. Disposition
   - Inform officer of what action needs to be taken next on their part
   - Identify items that have been retained frozen in laboratory

16.7 Theory

16.7.1 The laboratory report communicates to its reader the analytical results, conclusions of the analyst, and statistical statements supporting that conclusion.

16.7.2 The report is the culmination of the testing process in which scientific data is compiled into a format easily understandable by a non-scientific recipient. All results or conclusions in the formal report must be supported in the case notes such that a supervisor or independent forensic scientist would be able to draw the same conclusions after reviewing the detailed case notes.

16.7.3 Assessment Questions

- Which statements are commonly used to report a mixture?
- How is an amended report written? Under what circumstances would an amended report be required? What additional items does an amended report require?
- What information is required by the Quality Assurance Standards to be in a forensic DNA report?
- When is it necessary to include a statistical interpretation in your report?
- How would you explain the following to a jury? (You may want to develop analogies to aid in your explanations.)
    - The steps in the DNA analysis process
    - DNA mixtures (what they are and how you detect one)
    - The meaning of the statistics statement
    - The reason the statistics can be so rare by using only 24 markers?
- What information would you want to convey to qualify you as an expert on the stand?
- Why is it important to state for the record that our analysis is “PCR based STR typing?”
- Suggest and discuss four areas you might expect to be challenged about in court.
- Discuss with your trainer the advantages and disadvantages of your voice elements (volume, pitch characteristics, answering every statement with a question, dropping at end of statement (timbering). Discuss court appearance and appropriate dress.

16.8 Readings

16.8.1 See Required Reading Log – Module 15: DNA Report Writing

16.9 Practice

16.9.1 Observation

16.9.1.1 The trainee will observe qualified analysts drafting DNA reports in LIMS once data has been analyzed.

16.9.2 Training Exercises

16.9.2.1 The trainee will be provided with a minimum of 10 cases for which data has been generated and a report needs to be drafted using LIMS. Using the DNA SOP and

instruction from the trainer, or an assigned analyst mentor, the trainee will interpret the results and prepare a report for each case.

16.9.2.2 These cases will be re-analyzed by a qualified DNA analyst who will take ownership and issue the DNA report.

16.9.2.3 The analyst who took ownership of the trainee's case, shall provide feedback to the trainee and the trainer with a written summary of any findings to help assess the trainee's ability to compile a case file, interpret the data, and generate a DNA report.

16.10 Conclusion

16.10.1 Competency Test

16.10.1.1 Written test

16.10.1.1.1 A written test will be administered by the trainer that will incorporate DNA Training: Interpretation, Statistics, CODIS, and Report Writing/Case Review

16.10.1.1.2 Multiple shorter tests, broken down by learning concept, can be administered in place of one all-encompassing written test.

16.10.1.1.3 A hardcopy of the test(s) will be stored within the Training Binder.

16.10.1.2 Practical DNA Report Writing Case

16.10.1.2.1 Successful completion of a practical case (mock or casework case) is required prior to independent participation in casework.

16.10.1.3 The trainee will be authorized to perform independent casework via a signed authorization memo.

17 Module 16: DNA Case Review Mentorship Program

17.1 Duration

17.1.1 Approximately one to two months

17.2 Purpose

17.2.1 The purpose of this mentorship is to allow the newly qualified analyst to write cases and gain experience, but continue to be mentored in their writing skill level. As techniques are added to an experienced analyst's repertoire, he/she is not required to reenter the case mentorship program.

17.2.2 To provide guidelines for DNA casework technical reviews, administrative reviews, and quality reviews.

17.2.3 To become familiar with the technical review process for DNA cases.

17.3 Prerequisite

17.3.1 Module 15: DNA Report Writing

17.4 Theoretical Objectives

17.4.1 Trainee will be able to:

- Describe the different tasks within a technical, administrative, and quality case review for DNA casework.

17.5 Practical Skills

17.5.1 Trainee will be able to:

- Technically review a DNA case in regards to STR analysis, interpretation, and report writing.
- Administratively review a DNA case.
- Perform a Quality Review on the DNA report.

17.6 Module Outline

I. Technical Review
II. Documentation
    - Rough Draft or Final Report
    - Appropriate Checklist
    - Chronological Order
III. Administrative Review
IV. Quality Review

17.7 Theory

17.7.1 The technical review is an evaluation of documentation to check for consistency, accuracy, and completeness. The review is an evaluation of reports, notes, data and other documents to ensure there is appropriate and sufficient basis for the scientific conclusions. If applicable, CODIS profiles identified for upload are evaluated for eligibility and completeness. The technical review must be conducted by a second qualified analyst.

17.7.2 The administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.

17.7.3 The Quality Review is an evaluation of the physical report. If applicable, CODIS entry is verified.

17.7.4 Assessment Questions

- How does the technical review differ from the administrative review?
- How are defects discovered during the technical/administrative or quality reviews addressed?

17.8 Readings

17.8.1 None

17.9 Practice

17.9.1 Report Writing Mentorship

17.9.1.1 The newly qualified analyst will write DNA reports for a minimum of 50 cases.

17.9.1.2 The newly qualified analyst must have their first 50 cases technically reviewed by a supervisor or FBIO management approved analyst.

17.9.1.3 Keep track of types of cases, offenses, number of samples, number of mixtures, number of statistics, number of comparisons performed on the 50 cases.

17.9.1.4 Keep copies of the TR/AR checklists for each of the 50 cases.

17.9.2 Technical Review/Admin Review Exercises

17.9.2.1 The Mentorship cases of the newly qualifed analyst must be complete.

17.9.2.2 The newly qualified analyst will perform the technical review and administrative review on a minimum of 10 cases.

17.9.2.3 These cases will be technically and administratively reviewed again by a qualified DNA analyst.

17.9.2.4 The experienced DNA analyst must provide the trainee and the trainer with a written summary of any findings to help assess the trainee's ability to review a DNA case.

17.10 Conclusion

17.10.1 The trainee will be authorized to perform technical review and administrative review via a signed authorization memo.

17.10.2 Approval by Technical Leader to perform Quality Review.