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REVISION HISTORY

Effective Date	Brief Description of Change(s)
11/04/2019	Original Issue Previous revision history for individual chapters included in archived documents

01 OVERVIEW

DNA-TM-01-01 OVERVIEW OF DNA TRAINING PROGRAM

1 Introduction

Individuals employed by the Texas Department of Public Safety as DNA analysts must meet specific qualifications as outlined in the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories before being qualified to perform work. The qualifications consist of educational requirements and forensic experience requirements. These qualifications are reviewed by the Technical Leader prior to beginning work in the DNA section.

This training manual addresses training requirements for processing forensic biology evidence, male screening, DNA analysis, DNA interpretation, and DNA support. After completing the required training units, individuals may complete certain modules in order to be approved for work in specific processes in the DNA section based on the current needs of the laboratory. It is not required or expected that an individual complete the entire manual as long as they complete the portions required to demonstrate competency has been achieved for the processes in which they participate. Additionally, unless noted in the prerequisites section, it is not required that the manual be completed in any particular order.

This manual is used to train Biology screeners, DNA technicians and DNA analysts. The manual is intended for use by new personnel and any personnel attempting to become qualified in a procedure for which they are not currently qualified.

1.1 Definition of Personnel

A. Biology screener/Male Screening preparation: includes individuals that perform non-DNA analysis on forensic evidence.

   1. These individuals may test the evidence for the presence of body fluids, collect samples, prepare evidence for DNA processing, issue reports on the results of their analysis, and perform technical review for other screeners performing similar functions.

   2. Because these individuals do not perform DNA analysis, they are not audited to the FBI's Quality Assurance Standards, but are audited to the standards of the accrediting body.

B. DNA Technician: includes individuals that perform analytical DNA techniques (extraction, quantification, amplification, and electrophoresis) on forensic samples under the direction of a qualified DNA analyst.

   1. These individuals do not interpret DNA data, reach conclusions on typing results, or prepare final reports on DNA analysis.

   2. If they perform only non-DNA analysis on forensic evidence, they are audited to the standards of the accrediting body.

   3. If they perform analytical DNA techniques, they are audited to both the FBI's Quality Assurance Standards and the standards of the accrediting body.

C. DNA Analyst: includes individuals that conduct and/or direct the analysis of forensic samples, interpret data, and reach conclusions on DNA data.

   1. These individuals issue reports on the results of analysis and perform technical review for other analysts performing similar functions. This includes those

individuals qualified in interpretation of quantification results for use in the Male Screening process.

2. DNA analysts are audited to both the FBI's Quality Assurance Standards and the standards of the accrediting body.

1.2 Forensic Training/Experience Requirements

A. 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 work using DNA technology (this includes use of quantification for the purpose of Male Screening).

B. All personnel shall have successfully completed a qualifying test before beginning mentored/supervised work in their category of work/relevant testing procedure.

2 Purpose

A. The DNA training manual is designed to provide the trainee with sufficient background, laboratory skills, education, and supervised hands-on experience to competently perform work in the DNA section with minimal supervision and meet national standards.

B. The training program may be adjusted on a case-by-case basis to accommodate trainees that have prior forensic DNA experience, skills, and knowledge. Any adjustments to the training program (i.e., program content or requirements) are justified in writing in the trainee's notebook and approved by the System Quality Manager and relevant Technical Leader. Specific modifications to the training program should be indicated on the applicable training checklist.

3 Program Format

A. The training program is divided into seven units, each consisting of a set of modules: General Laboratory Training Unit, Introductory Forensic Biology/DNA Unit, Biological Evidence Screening Unit, Male Screening Unit, Analytical DNA Unit, DNA Interpretation Unit, and DNA Support Staff Technician Unit.

B. The General Laboratory Training Manual and the Introductory Forensic Biology/DNA Unit are required for all new personnel in the DNA section.

1. <u>The General Laboratory Training Manual</u> introduces the trainee to general laboratory practices, forensic science, quality assurance, general laboratory safety, legal issues, LIMS system, and department and laboratory policy.

   a) The Fundamentals Unit within the General Laboratory Training Manual must be completed in its entirety before the trainee can handle any unknown substances in the laboratory per the Safety Manual.

   b) The General Laboratory Training Manual Legal Unit must be completed before approval for mentored/supervised work is granted.

2. <u>The Introductory Forensic Biology/DNA Unit</u> introduces the trainee to the history of Forensic Biology/DNA, issues affecting quality, basic equipment use/quality control, and proper molecular biology laboratory technique.

   a) The anticipated duration of this unit is no longer than 1 month.

   b) Completion of the Introductory Forensic Biology/DNA unit (as documented by the trainer's approval) is required prior to beginning the Biological Evidence

Screening, Male Screening, Analytical DNA, DNA Interpretation, and DNA Support Staff Technician units.

C. The Biological Evidence Screening Unit, the Male Screening Unit, the Analytical DNA Unit, the DNA Interpretation Unit, and the DNA Support Staff Technician Unit are intended to be modular in nature.

1. The trainer, after consultation with the Technical Leader, has the option to assign the trainee single modules in these units for completion. This allows the trainee to become qualified in specific categories of work/relevant testing procedures as best serves the needs of the laboratory in which they are stationed.

2. Biological Evidence Screening Unit introduces the trainee to sample preparation, stain identification and documentation, case evaluation, report writing, and technical review.

   a) *This unit is intended for use in training personnel in Biological Evidence screening.*

   b) *The anticipated duration of this unit is 2-4 months.*

3. Male Screening Unit introduces the trainee to cutting of samples for male screening, performing DNA quantification for the purpose of male screening, and interpretation of quantitation results for purposes of male screening.

   a) *This unit is intended for use in training personnel in preparation of samples for Male Screening, training DNA technicians and DNA analysts in the analytical techniques completed prior to Male Screening interpretation, and training DNA analysts in interpretation of Male Screening data.*

   b) *The anticipated duration of this unit is 2-4 months.*

4. Analytical DNA Unit introduces the trainee to analytical DNA techniques (extraction, quantification, amplification, and electrophoresis), and robotics.

   a) *This unit is intended for use in training DNA technicians and DNA analysts in the analytical techniques that are completed prior to DNA interpretation.*

   b) *The anticipated duration of this unit is 3-6 months.*

5. DNA Interpretation Unit introduces the trainee to STR analysis and interpretation, statistics, DNA case evaluation, report writing, and technical review.

   a) *This unit is intended for use in training DNA analysts in interpretation of DNA data.*

   b) *The anticipated duration of this unit is 6-8 months.*

6. DNA Support Staff Technician Unit introduces technician trainees to preparation and quality control checks of reagents commonly used in biological evidence screening, and preparation of reagents commonly used for analytical DNA processes.

   a) *This unit is intended for use in training technicians on how to prepare and quality check reagents commonly used in biological evidence screening. It is also intended for use in training technicians that do not participate in casework activities on how to prepare reagents commonly used in DNA analytical processes.*

   b) *The anticipated duration of this unit is no longer than two weeks.*

D. Samples listed in the competency sections for each unit will be in addition to and separate from any samples worked during the practice portion of the training modules within each unit.

1. Competency samples from the DNA extraction competency test may be used to satisfy competency requirements for DNA quantification, DNA amplification, capillary electrophoresis, and robotic platform.

2. The same sample may be used multiple times as a competency test sample for these modules.

E. The requirement for successful completion of written examinations given as part of training listed in the DNA training manual is a score of 75% or greater.

If a score of less than 75% is obtained, the remediation process outlined in the CLS Employee Training Program chapter will be followed.

4 Safety

A. Specific safety precautions are described within each module of the training manual.

B. In general, body fluids and other biological material encountered during training in Biology/DNA may contain infective agents. Use universal precautions during evidence handling.

5 Responsibilities

5.1 Technical Leader and/or Supervisor Responsibilities

A. The Technical Leader is responsible for overseeing all training in the DNA Section.

B. The Technical Leader may assign a trainer(s) to the trainee for general laboratory assistance, instruction in procedures, and laboratory practicals and assessments.

C. Meetings between the trainee, the trainer, Technical Leader, and/or Supervisor should be held routinely in order to evaluate the trainee's progress, plan future study/practical assignments and discuss any deficiencies which require additional training.

5.2 Trainer Responsibilities

A. The trainer(s) conducts training on assigned modules, reviews training notebook, training records, and completion of training requirements.

The trainer will be qualified by DPS and proficiency tested in the category of work/relevant testing procedure for which they provide training.

B. The trainer(s) also plans, conducts and evaluates mock trial.

C. The trainer(s) recommends that the examiner be approved for mentored/supervised work to the Technical Leader.

5.3 Trainee Responsibilities

A. During the training program, the trainee is responsible for keeping detailed records in a training notebook that documents completion of training requirements (including testimony where applicable), required exercise/practical result summaries, competency testing (including report writing wherever it is a job responsibility), and other records as required.

1. Completion of modules and/or practicals will be recorded on the appropriate unit training checklist (LAB-TM-DNA-01, LAB-TM-DNA-02, LAB-TM-DNA-03, LAB-TM-DNA-04, LAB-TM-DNA-05or LAB-TM-DNA-06.)

2. The following is a list of items maintained in the training notebook by the trainee:

   *a) Training Record (LAB-303) (**optional**)*

   b) List of any in-house training videos and lectures attended that are not already listed on the appropriate unit training checklist.

   c) Sample identification results and other practical exams

   d) Competency tests and results

   *e) Validation results or special project assignments with summary reports (**optional**)*

   f) Written exams, oral exam comments, mock trial documentation (LAB-313 and LAB-314)

   g) Summary of cases reviewed (do not include case number or any identifying information)

   h) Courtroom testimony attended and observations (LAB-313 and LAB-314) (do not include case number or any identifying information)

   i) Workshop or lecture certificates (copy)

B. The training program covers information that requires the trainee to keep up with reading assignments on a self-study basis.

C. The trainee is responsible for informing his/her trainer or Technical Leader when problems arise at any time during the training period.

D. The trainee will document their progress through the training record and successfully complete all exams and competency tests.

6 Review and Authorization

6.1 General Laboratory Training Unit and Introductory Forensic Biology/DNA Unit

General Laboratory Training Unit and Introductory Forensic Biology/DNA Unit requirements will conclude when the following are met:

A. The trainee successfully completes General Laboratory Training Manual Fundamentals Units

B. The trainee successfully completes General Laboratory Training Manual Legal Units

C. Trainee successfully completes the Introductory Forensic Biology/DNA Unit.

D. Completion of the general laboratory training and Introductory Forensic Biology/DNA unit is documented via the Certificate of Completion (LAB-308) with recognition by the Laboratory Director.

6.2 Biological Evidence Screening Unit

A. Biological evidence screening unit training will conclude with examiner approval to conduct mentored/supervised work when the following are met:

1. Competency samples for the category of work/relevant testing procedure are correctly analyzed.
2. The trainee successfully completes a written exam(s) covering material presented in the modules relevant to the testing methods for which the trainee seeks qualification.
3. Successful completion of mock case(s) which would include examination of evidence relevant to the testing methods for which the trainee seeks qualification.
4. Successful completion of mock trial.

B. Additional optional comprehensive oral board on the topics covered in the Biological Evidence Screening Unit to cover the spectrum of anticipated work to be performed.

C. Additional optional exercises covering laboratory policy: case acceptance, triaging cases, and prioritizing evidence.

D. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.

E. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

The employee is considered a qualified analyst/technician in the relevant category of testing once they have received authorization to conduct work from the Laboratory Director. This includes authorization to conduct validation and quality control work in their relevant category of testing.

F. The newly qualified analyst/technician is enrolled in the proficiency testing program once the Work Authorization form (LAB-309) has been completed.

6.3 Male Screening Unit

A. Male screening unit training will conclude with examiner approval to conduct mentored/supervised work when the following are met:

1. Competency samples for the category of work/relevant testing procedure are correctly analyzed.
2. Completion of six months of forensic DNA laboratory experience for male screening interpretation only. This may run concurrently with training in the Biology Screening, Analytical DNA, or DNA Interpretation unit.
3. Successful completion of an exam to cover the spectrum of anticipated work to be performed (including questions relevant to possible testimony) for those training only in Male Screening Preparation. This exam serves as a mock trial in Male Screening Preparation and is only required if the trainee is not also qualifying concurrently in Male Screening Process. Completion of LAB-313 Testimony Technical Review is required.
4. Successful completion of mock trial for those training in Male Screening Process.

B. Additional optional comprehensive oral board on the topics covered in the Male Screening Unit to cover the spectrum of anticipated work to be performed.

C. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.

D. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

E. The newly qualified analyst/technician is enrolled in the proficiency testing program once the Work Authorization form (LAB-309) has been completed.

6.4 Analytical DNA Unit

A. The Analytical DNA Unit training will conclude with examiner approval to conduct mentored/supervised work when the following are met:

1. Competency samples for the category of work/relevant testing procedure are correctly analyzed.
2. Successful completion of at least one mock case that includes processing of DNA from evidence relevant to the analytical DNA methods for which the trainee seeks qualification.
3. Successful completion of a comprehensive exam to cover the spectrum of anticipated work to be performed (including questions relevant to possible testimony). This exam serves as a mock trial in analytical DNA methods and is only required if the trainee is not also qualifying concurrently in DNA Interpretation. Completion of LAB-313 Testimony Technical Review is required.

B. Additional optional comprehensive oral board on the topics covered in the Analytical DNA Unit to cover the spectrum of anticipated work to be performed.

C. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.

D. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

E. The newly qualified analyst/technician is enrolled in the proficiency testing program once the Work Authorization form (LAB-309) has been completed.

6.5 DNA Interpretation Unit

A. DNA interpretation unit training will conclude with examiner approval to conduct mentored/supervised work when the following are met:

1. Competency samples for the category of work/relevant testing procedure are correctly analyzed.
2. Successful completion of six months of forensic DNA laboratory experience. This may run concurrently with training in the Biology screening, Male screening, or Analytical DNA unit.

3. Successful interpretation of data from at least one mock case which includes mixture data.
4. Successful completion of mock trial.

B. Additional optional comprehensive oral board on the topics covered in the DNA Interpretation Unit.

C. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.

D. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

E. The newly qualified analyst is enrolled in the proficiency testing program once the Work Authorization form (LAB-309) has been completed.

6.6 DNA Support Staff Technician Unit

A. DNA support staff technician unit training will conclude with technician authorization to prepare (and quality check as appropriate) reagents when the following are met:

Competency samples for the category of work/relevant testing procedure are completed correctly.

B. The training notebook and other training records documenting completion of training requirements are reviewed by the trainer and the Technical Leader. Quality Assurance and the Section Supervisor (if applicable) may also review the training notebook and other training records.

C. The training notebook is submitted along with a Work Authorization form (LAB-309) to System QA for review and Laboratory Director authorization.

D. There is no proficiency or mentored/supervised work requirement for support staff technicians who only participate in the preparation of reagents.

6.7 Mentored/Supervised Work Requirements

A. Process

1. Upon completion of training requirements, analysts/technicians new to DPS will enter a period of mentored/supervised work authorized by the Laboratory Director.
2. Those analysts/technicians previously qualified to conduct work at a DPS Crime Laboratory may either be authorized for an abbreviated period of mentored/supervised work or authorized for independent work by the Quality Manager.
3. At least one mentor that was either previously qualified or is currently qualified in the category of work that they are overseeing is assigned to the new examiner/technician.
4. Mentors must make a note in the case record for mentored/supervised cases to indicate that they concur with the examination results.
5. Examiners/technicians will document the cases on which mentored/supervised work was performed using LAB-307 (Supervised Case Log) or electronic equivalent.

6. Once all mentored/supervised work requirements are successfully completed, the mentor(s) can recommend the examiner/technician for independent work to the Technical Leader. The authorization for independent work is granted through completion of the Work Authorization form (LAB-309) by the Quality Manager and Technical Leader. The Section Supervisor (if applicable) may also sign this form; however, this signature is not required.

B. Mentored/Supervised Work Requirements

1. For Biological Evidence screening: successful completion of a minimum of 10 screening and/or body fluid identification cases.

   a) *Level 1: Mentor physically watches examiner complete all aspects of case handling and analysis (at least 5 cases)*

   b) *Level 2: Mentor examines sperm slides and ABAcard test results, etc. (the remainder of the cases)*

2. For Male Screening Preparation: successful completion of a minimum of 10 cases.

   Note: Mentored/supervised work for Male Screening Preparation is not required for examiners who were previously qualified for independent work in Biological Evidence Screening.

   a) *Level 1: Mentor physically watches examiner complete all aspects of case handling (at least 5 cases)*

   b) *Level 2: Mentor verifies labeling and itemization of evidence (remainder of the cases)*

3. For Male Screening Process (plate processing and interpretation): successful completion of a minimum of 1 full plate or 10 cases.

   Note: Mentored/supervised work for Male Screening Process is not required for examiners/technicians who were previously qualified for independent work in the methods used for completion of the process (e.g. quantification, liquid handling robots) or for analysts that were previously qualified in interpretation of quantification results

   a) *Plate processing: Mentor physically watches examiner/technician complete all aspects of case handling and analysis (at least 1 full plate of 10 cases).*

   b) *Interpretation: Mentor is consulted during interpretation and views the report prior to analyst turning it in for technical review (10 cases).*

4. For Analytical DNA: successful completion of a minimum of 10 cases for DNA analysis.

   a) *Level 1: Mentor physically watches analyst/technician complete all aspects of case handling and analysis (at least 1 case).*

   b) *Level 2: Mentor is consulted at each step of the analysis procedure prior to analyst/technician proceeding with that step (remainder of the cases).*

5. For DNA Interpretation: successful completion of a minimum of 10 cases for DNA interpretation and reporting.

   a) *Level 1: Mentor physically watches analyst complete all aspects of case interpretation and reporting (at least 1 case).*

b) Level 2: Mentor is consulted during interpretation and views the report prior to analyst turning it in for technical review (remainder of the cases).

6.8 Evaluation of Training

The trainee will complete an evaluation of each module content, the unit content, or the overall program including the trainer using the LAB-304. The trainee and trainer will complete a checklist and sign-off sheet.

DNA-TM-01-02 DNA TRAINING UNITS MATRIX

1 Color Key

☒ REQUIRED

■ RESTRICTED – NOT ALLOWED

■ CONDITIONAL – JOB FUNCTION DEPENDENT

BLANK SPACE = NOT REQUIRED

2 Matrix

	MS	BES	DNA	TECH
General Training Manual				
Fundamentals Unit	☒	☒	☒	☒
Forensic Legal Unit	☒	☒	☒	☒
DNA Training Manual				
Overview	☒	☒	☒	☒
Introductory Forensic Biology/DNA Unit				
Introduction to Forensic Biology and DNA – History of the Disciplines	☒	☒	☒	☒
Basic Equipment Use and Issues Affecting Quality	☒	☒	☒	☒
Biological Evidence Screening Unit				
Blood Screening		■		■
Semen Identification and Determination		■		■
Forensic Biology Report Writing and Technical Review		☒		■
Male Screening Unit				
Male Screening – Sample Preparation	☒			■
Male Screening Process	■			■
Male Screening Report Writing and Technical Review	■			■
Analytical DNA Unit				
Manual DNA Extraction			■	■
Manual Quantification			■	■
Manual Amplification			■	■
Liquid Handling Robotics	■		■	■

	MS	BES	DNA	TECH
Analytical DNA Unit				
Automated DNA Extraction - PrepFiler			■	■
Automated DNA Extraction – Qiagen EZ1			■	■
Capillary Electrophoresis Instrument Operation and Troubleshooting			■	■
DNA Interpretation Unit				
Theory of Analytical DNA			■	■
Data Analysis and Sample Characterization			☒	■
Manual Mixture Interpretation, DNA Comparison, and Match Statistics			☒	■
STRmix Mixture Deconvolution Software and Likelihood Ratios			☒	■
CODIS			☒	■
DNA Report Writing and Case Review			☒	■
Legacy Amplification Kit Data: Interpretation and Review			■	■
DNA Support Staff Technician Unit				
Reagent Preparation and Quality Control for Technicians				■

3 Designation Key

MS Male Screener

BES Biology/Serology Screener

TECH DNA Technician/DNA Analyst (non-reporting option)

DNA DNA Analyst

02 INTRODUCTORY FORENSIC BIOLOGY/DNA UNIT

DNA-TM-02-01 INTRODUCTION TO FORENSIC BIOLOGY AND DNA – HISTORY OF THE DISCIPLINES

Purpose Enable a trainee to communicate using appropriate forensic biology and DNA terminology. Educate a trainee on the earlier procedures and advances in forensic biology and DNA analysis.

Prerequisite None

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How to understand and use proper biological terminology for successful communication in both the forensic field and in the courtroom.

B. The history of the developments and past methods in forensic biology and human identification testing that provide a foundation to the concepts and processes used toward the individualization of a forensic stain and subsequent forensic DNA testing.

1.2 Practical

Following the completion of training, the trainee will be able to:

A. Define and understand basic genetic, biological, and immunological terms.

B. Define and understand basic heredity and forensic DNA typing terms.

C. Discuss from a historical point of view the major developments in forensic biology.

D. Discuss advances in forensic biology and applying forensic biology principles to body fluid identification.

E. Discuss the major developments in forensic DNA from a historical point of view.

F. Discuss the basis for applying population statistics to antigen, enzyme, and protein typing results.

G. Describe the limitations of antigen, antibody, isoenzyme, and protein analysis.

H. Describe the limitations of DNA analysis.

I. Discuss serological techniques used in the trainee's respective laboratory prior to the implementation of DNA analysis.

J. Discuss DNA techniques used in the trainee's respective laboratory prior to the implementation of short tandem repeat analysis.

K. Discuss the principles of the forensic serology theories when applying body fluid identification.

L. Be aware of available forensic DNA typing systems (e.g. RFLP, PCR-based, sequencing-based such as mitochondrial and SNPs).

2 Training Outline

2.1 Lesson Plan

A. Introduction to forensic biology and DNA
   1. Definition, importance, legal value
   2. Basic biochemistry (amino acids, proteins, enzymes)
   3. Basic genetics (mitosis, meiosis, mutations, polymorphisms)
   4. Basic immunology (antigen/antibody concepts)
   5. The scientific basis of DNA typing
   6. Other areas utilizing DNA typing techniques

B. History of forensic biology
   1. Principles of Heredity
      a) genes
      b) chromosomes
      c) locus
      d) alleles
      e) genotype
      f) phenotype
      g) homozygous
      h) heterozygous
   2. Early blood and semen discoveries
   3. Genetic markers of the red cell
      a) Red cell antigens
      b) Red cell isoenzymes and proteins
   4. Genetic markers of the plasma
      a) Plasma ABO antibodies and antigens
      b) Plasma proteins
   5. Genetic markers of semen and saliva
      a) Soluble antigens
      b) Isoenzymes in semen (PGM)
   6. Other body fluids
   7. Long-term storage of evidence stains

C. History of DNA
   1. Overview of forensic DNA typing systems
      a) RFLP
      b) PCR – HLA Dqα and D1S80

c) *PCR – Autosomal STRs, Mini-STRs, Y-STRs*

d) *Sequencing - Mitochondrial DNA, SNPs*

2. Procedures for forensic DNA analysis

a) *Isolation of DNA*

b) *Determining quality and quantity of DNA*

c) *PCR amplification*

d) *Analysis of PCR product*

3. Major historical events in forensic DNA typing

D. Legal aspects

1. Senate Bill 1636

a) *Non-prosecution cases*

b) *No-bill cases*

c) *Plea cases*

2. Senate Bill 1292

2.2 Required Readings

A. Introduction to Forensic Biology and DNA

1. Houck, Max and Siegel, Jay. Fundamentals of Forensic Science. 2nd Ed. Academic Press. 2010. Ch. 10 and 11, pp 229-282.
2. Taupin JM and Cwiklik C. Scientific Protocols for Forensic Examination of Clothing. CRC Press LLC. 2011. Sections: 1.1-1.5, 2.1-2.4, 2.6-2.8, 3.1-3.3, and 7.
3. Proper evidence storage: Code of Criminal Procedure Article 38.43.
4. Government Code 411.053.
5. Texas Administrative Code Title 37, Part 1, Chapter 28, Subchapter K, Rule 28.182.
6. Butler, John M. 2010. Fundamentals of Forensic DNA Typing. Elsevier, Inc. Ch. 1-2.
7. National Research Council. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press. Ch. 1-2.
8. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

a) *Overview;*

b) *Standard Abbreviations List;*

c) *Glossary of Terms;*

d) *Relevant Forms.*

B. History of Forensic Biology

1. Eckert WG. Introduction to Forensic Science. 2nd Edition. 1997. pp.240-244.
2. Saferstein R. Criminalistics: An Introduction to Forensic Science, 7th Edition. Prentice Hall Inc. 2001. pp 320-335.

C. History of Forensic DNA

1. Butler, John M. 2010. Fundamentals of Forensic DNA Typing. Elsevier, Inc. Ch. 3.
2. Texas Department of Public Safety. Human Identity Testing Chronology (handout) – (most recent version).
3. Moretti TR, et al. Validation of Short Tandem Repeats (STRs) for Forensic Usage: Performance Testing of Fluorescent Multiplex STR Systems and Analysis of Authentic and Simulated Forensic Samples. J For Sci. 2001. 46, pp. 647-660.
4. Taupin, JM. Introduction to Forensic DNA Evidence for Criminal Justice Professionals. CRC Press. Ch. 3.

D. Legal Aspects

1. Senate Bill 1292, 2013. Texas Legislature (Code of Criminal Procedures Article 38.43)
2. Senate Bill 1636, 2011. Texas Legislature (Government Code 420)

3 Assessment

3.1 Competency and Qualifying Examination

The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

3.2 Evaluation of Training

The trainee and trainer will complete the Introductory Forensic Biology/DNA Unit Training Checklist (LAB-DNA-TM-01).

DNA-TM-02-02 BASIC EQUIPMENT USE AND ISSUES AFFECTING QUALITY

Purpose Familiarize trainee with general equipment available in the laboratory. Enable trainee to safely operate, calibrate, clean and maintain laboratory equipment. Orient and acquaint trainee with precautions taken in the laboratory to prevent contamination, sample switch, and loss of DNA.

Prerequisite GLT Fundamentals Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How the proper use and maintenance of laboratory equipment is essential to good laboratory practice. All laboratory equipment must be cleaned, calibrated, and used correctly to ensure its reliability.

B. How proper documentation of equipment calibrations and maintenance is crucial to demonstrating quality control in the laboratory. Maintenance and calibration guidelines must be written and well understood by all users.

Note: This module is intended to be a general overview of available laboratory equipment. More detailed instruction on specific instruments or equipment will be presented in subsequent modules.

C. The impact of producing a reliable and quality result in the laboratory.

D. How an analyst's and laboratory's reputation relies on the integrity and quality of the work product.

E. Factors affecting quality that require stringent measures and precautions to be practiced at all steps of samples handling such as:

1. Contamination (the process in which DNA not originally present in the sample is introduced into a sample.)
2. Sample switches (situations when the analyst inadvertently replaces one sample with another.)
3. Loss of DNA (occurs naturally over time but can also occur if evidence is mishandled.)

***Note**: In some cases, there is no additional evidence available for re-analysis.*

1.2 Practical

Following the completion of training the trainee will be able to:

A. Distinguish between different types of microscopes.

B. Describe two types of displacement pipettes.

C. Outline the routine maintenance and calibration procedures for equipment in the laboratory.

D. Understand the use of types of quality controls for all equipment used in the laboratory.

E. Describe chemicals and environmental insults promoting the degradation and loss of DNA.

F. Understand contamination, possible sources of contamination in the laboratory, and the steps necessary to prevent contamination within each dedicated work area.

G. Discuss possible implications of contamination, sample switches and loss of DNA.

H. Describe actions taken to preserve DNA.

I. Describe the necessary precautions taken to prevent sample switches.

J. Discuss the proper response to contamination or a sample switch.

K. Discuss ways to record contamination events.

L. Describe different parts of a Quality Incident/Action Plan (QI/QAP).

M. For the specific equipment in the laboratory, the trainee will be able to:

1. Weigh varying quantities of substances on an analytical balance. Trainee will demonstrate proper cleaning procedures for spilled chemicals on the balance pan.
2. Choose proper pipettes and tips appropriate for the volume to be measured and demonstrate proper technique in sample uptake, dispensing, and tip ejection. Trainee will be able to clean pipettes.
3. Demonstrate proper technique in loading and running centrifuges and dismantle the centrifuge for cleaning and disinfection.
4. Properly package items for autoclaving and load, operate, empty and clean the autoclaves.
5. Become familiar with the wet/dry incubators, their operation and applications, checking and changing temperatures and routine cleaning.
6. Perform general cleaning and modified Kohler illumination on microscopes; dial between objectives. **Optional**: set up microscope for phase contrast microscopy.
7. Complete relevant documentation pertaining to equipment use, quality control checks and calibrations.
8. Prepare a 1:10 bleach solution and properly clean work surfaces.
9. Properly cleanse forceps, scissors, and other tools.
10. Properly dispose of used or contaminated materials and supplies.
11. Demonstrate proper handling of samples, handling of microcentrifuge tubes, proper pipetting techniques, and use of reagents.

2 Training Outline

2.1 Lesson Plan

A. Introduction to Basic Equipment in the laboratory

1. Reference materials
2. Location of available equipment
3. Equipment maintenance
4. Calibration log book(s)
5. Critical instruments and equipment

6. Quality control documentation

B. Pipettes

1. Instruction on choice of size and operation of different pipettes (mechanical and electronic, if available)
2. Maintenance and cleaning
3. QC specifications, calibration, performance check, and documentation

C. Balances

1. Instructions on operation, maintenance, and cleaning
2. QC specifications, calibration and documentation

D. Wet/Dry Incubators

1. General biological wet/dry incubators
2. DNA dedicated wet/dry incubators
3. Maintenance and cleaning
4. QC specifications and documentation

E. Microscopes

1. Types and uses of compound light microscopes in the laboratory
   a) Stereoscope
   b) Comparison scope
   c) Brightfield microscopes
2. General care and maintenance of microscopes
3. Microscope adjustment – modified Kohler illumination

F. Autoclaves

1. Instructions on usage applications (sterilization of solutions, biological waste, proper labeling and disposal of biological waste, etc.), operation and cleaning
2. QC documentation regarding sterilization of biological waste

G. UV Crosslinker (if available in trainee's lab)

1. Instructions on usage applications
2. QC documentation

H. Refrigerators/Freezers

1. Location and storage application
2. Temperature specifications and alarm device
3. Cleaning (routine and major) and maintenance
4. QC documentation

I. Centrifuges (microcentrifuge and larger volume centrifuge)

1. Instruction on operation, maintenance, and cleaning (disinfection)

2. QC specifications, calibration and documentation

J. Thermometers (standard and digital)
   1. Instructions on handling, reading, and maintenance
   2. QC specifications, calibration and documentation
   3. NIST traceable documentation

K. Chemical and Biological Hoods
   1. Laminar Flow Biological Safety Cabinet
      a) Principles of operation
      b) Uses of laminar flow hoods
      c) Certification and maintenance
   2. Chemical Fume Hoods
      a) Principles of operation
      b) Uses of chemical fume hoods
      c) Maintenance of chemical fume hoods

L. Alternate Light Sources
   1. Principles of operation
   2. QC specifications and documentation

M. DNA degradation
   1. Chemicals, detergents
   2. UV light, sunlight
   3. Heat, humidity
   4. Bacteria, microorganisms
   5. Endo and exonucleases
   6. Biological fluid mixtures
   7. Shear forces
   8. Time

N. Preserving stains/DNA
   1. Drying
   2. Refrigeration or freezing
   3. EDTA additive
   4. FTA paper

O. Sources of laboratory contamination
   1. Human genomic DNA from the environment
   2. Sample to sample contamination

3. PCR product carry-over

P. Contamination detection measures

1. Negative control
2. Reagent blanks
3. Data Analysis Software tools
4. Review of case-specific information (do the results make sense?)

Q. Contamination prevention measures

1. Isolated and dedicated work areas
   - *a) Evidence handling work area*
   - *b) DNA extraction work area*
   - *c) PCR set-up work area*
   - *d) Amplified DNA work area*
2. Special precautions
   - *a) Dedicated equipment and lab supplies*
   - *b) Clean work surfaces and equipment*
   - *c) Clean cutting surface*
   - *d) Frequent glove changes*
   - *e) Cleansed scissors or disposable blades, forceps*
   - *f) Disposable pipette tips*
   - *g) Minimizing aerosols*
   - *h) Avoiding splashes*
   - *i) Autoclaving, sterilization*
   - *j) UV germicidal lamps*
   - *k) Separating evidence from reference samples*
   - *l) Single sample handling*
   - *m) Small sample size and/or number*
   - *n) Aliquot reagents*
   - *o) Use of decapper to open microcentrifuge tubes*
   - *p) Centrifuge tubes prior to opening*
   - *q) Use of appropriate PPE*
   - *r) Use of robotics/automation*

R. Response to contamination

1. Technical Leader Notification
2. Contamination log

3. Quality Action Plan
   a) *Root Cause Analysis*
   b) *Effect of the contamination*
   c) *Corrective Action*
   d) *Preventative Measures*

S. Sample switch prevention methods
1. Necessary Precautions
   a) *Establish a routine*
   b) *Minimize distractions*
   c) *Manage time effectively*
   d) *Incorporation of robotics/automation*
   e) *Label tubes appropriately for all sample transfers*
   f) *Ensure only the destination tube is open during insertion of the cutting/stain*
   g) *Pay attention to details (including checking and double-checking labels)*

      The analyst must verify sample information is consistent between originating item and destination container/tube/plate during sampling and all liquid transfers (during entire evidence handling process).
2. Considerations or Suggestions
   a) *Use colored tubes and/or colored labels*
   b) *Examine/extract suspect knowns at different times than victim or elimination samples*
   c) *Examine/extract victim and elimination samples first and suspect knowns later*
   d) *Alternate genders in the sequence of tubes. For example, male-female-male-female*
   e) *Place cuttings in tubes during biology or male screening*
   f) *Observation by a second analyst:*
      i. *Placement of cuttings into tubes*
      ii. *All tube transfers*

T. Sample switch detection measures
1. Reagent blank and amplification control results
2. Data Analysis Software tools
3. Review of case-specific information (do the results make sense?)

U. Responses to a sample switch
1. Technical Leader Notification
2. Quality Action Plan
   a) *Root Cause Analysis*

b) *Effect of the sample switch*

c) *Corrective Action*

d) *Preventative Measures*

V. Possible impacts of contamination and/or sample switches

1. Unnecessary use of resources
2. Loss or consumption of evidence
3. Investigation into proficiency of the analyst which may include removal from casework
4. Reputation
5. False inclusion or exclusion

2.2 Required Readings

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Scope (Statement of Quality Policy);*
   b) *Monitoring the Validity of Results;*
   c) *Audits;*
   d) *Validations and Performance Verifications;*
   e) *Laboratory Equipment;*
   f) *Document Management and Deviations);*
   g) *Quality Incident (QI) and Quality Action Plan Process (QAP);*
   h) *Evidence and Database Sample Integrity.*
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version)
   a) *Facilities;*
   b) *Validations;*
   c) *Analytical Controls;*
   d) *Response to Quality Issues;*
   e) *Instruments and Equipment;*
   f) *Forensic Biology and DNA Proficiency Instructions.*
3. User manuals for general laboratory equipment used by your lab. (Examples may include adjustable pipettes, balances, centrifuges, microscopes and stereoscopes, autoclaves, pH meters, vortexers, cameras, ALS, IR camera).
4. Microscope Cleaning and Maintenance-Carl Zeiss Microscopy Online Campus. (http://zeiss-campus.magnet.fsu.edu/print/basics/care-print.html).
5. Houck, Max and Siegel, Jay. Fundamentals of Forensic Science. 2nd Ed. Academic Press. 2010. pp 77-85.
6. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. pp. 1-10, 18-19, Ch. 7.
7. Cushwa WT and Medrano JF. Effects of Blood Storage Time and Temperature on DNA Yield and Quality. Biotechniques. 14. pp. 204-205

8. Farley, M and Harrington, J. Forensic DNA Technology. Lewis Publishers Inc. 1991. pp. 75-78, 186-187.

9. Gill P. Development of a Simulation Model to Assess the Impact of Contamination in Casework Using STRs. J For Sci. 49. pp. 485-491.

10. National Research Council. The Evaluation of Forensic DNA Evidence. National Academy Press. 1996. pp. 82-84.

11. Proceeding of the International Symposium on the Forensic Aspects of DNA Analysis. Federal Bureau of Investigation. Quantico, Virginia. 1989. pp. 14-15, 257-261, 267-271.

12. Senate Bill 03, 2001. Texas Legislature.

13. Federal Bureau of Investigation. Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (most recent version). Standards 3-10, 13-15.

14. Scientific Working Group on DNA Analysis Methods (SWGDAM). Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories. www.swgdam.org (most recent version).

15. Safety Data Sheets as applicable for each laboratory:
    - Bleach
    - DNA ZAP
    - DNA Away

3 Practice

3.1 Safety

A. Wear gloves and lab coats when working in the laboratory. Safety glasses or goggles and masks may be required during some operations. Be aware of potential hazards that exist in the laboratory.

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

C. Ensure that tubes in centrifuges are properly balanced and lid is securely closed before operating.

D. Handle thermometers carefully. Note: Mercury is a chemical hazard and should be handled as such whenever a thermometer is broken and mercury is released. (Refer to Safety Manual for handling mercury spills)

3.2 Standards, Controls, and Reagent Preparation

A. Controls

1. Calibration schedule of equipment; maintenance and calibration forms and log books; documentation requirements as indicated in the DNA SOP.

B. Reagent Preparation

1. 1:10 bleach solution (as applicable)

3.3 Equipment

Depending on availability in the trainee's laboratory:

- Balance
- Pipette tips
- Pipettors
- Centrifuge
- Microcentrifuge
- Autoclave
- Wet/dry incubators and shakers
- Compound microscope
- Forceps, scissors, and other utensils
- UV Crosslinker
- Thermometers
- Refrigerators/Freezers
- Chemical/Biological Hoods
- Alternate Light Source
- Microcentrifuge tubes (1.5 mL, 0.5 mL)
- Decapper
- Litmus paper or pH meter
- Unstained cloth or swabs
- Coin envelopes
- Bleach sprayers
- Sodium Chloride
- Citric Acid

3.4 Observed Performance

A. Trainer will demonstrate proper technique for operating laboratory equipment.

B. Trainer will give instructions on documentation of quality control procedures and calibration of pertinent equipment.

C. Trainer will demonstrate proper procedures for cleaning and preparing work surfaces and equipment in the dedicated work areas.

D. Trainer will give instruction on the cleansing of forceps and cutting tools, frequent changing of gloves and separation of evidence.

E. Trainer will give instructions on the supplies and reagents requiring sterilization.

F. Trainer will demonstrate proper handling of single samples, decapping microcentrifuge tubes, pipetting techniques and handling of reagents.

G. Trainer will give instruction on the disposal of used and contaminated supplies.

H. Trainer will discuss proper documentation of contamination and other quality events. Actual documentation may be used as examples. As an optional exercise, trainee may complete the sections of quality incident and/or contamination log documentation.

3.5 Supervised Performance

A. Exercise 1: Trainer will observe performance of operations and documentation as trainee completes the following exercise.

1. Weigh out some amount of sodium chloride or citric acid and add to 100 mL deionized water. Stir until it has dissolved. Measure the pH of the resulting solution.
2. Pipette the following volumes: 5.2 µL, 10.7 µL, 20 µL, 50 µL, 190 µL, 205 µL, 881 µL into individual microcentrifuge tubes. Clean each of the pipettes used in this exercise.
3. Demonstrate the procedure for centrifuging 2 microcentrifuge tubes for 15 seconds at 5000 x g; for centrifuging 7 tubes for 3 minutes at 1500 x g.
4. Demonstrate proper use of bleach sprayers and clean the work areas as applicable.
5. Properly clean the nearest microcentrifuge with the appropriate cleaning solution.

B. Exercise 2: Trainer will observe the trainee performing the following exercise.

1. Trainee will clean and prepare a work area in the laboratory.
2. Trainee will be given 5 unstained pieces of cloth/swabs, each inside a separate envelope.
3. Trainee will label 5 microcentrifuge tubes (1 through 5) and place them in a rack.
4. Trainee will be observed cutting portions from each of the samples and placing them into the appropriate tubes.
5. Trainee will be observed pipetting first 1000 microliters into each tube, and then pipetting 10 microliters into each tube.

4 Assessment

4.1 Competency and Qualifying Examination

The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

4.2 Evaluation of Training

The trainee and trainer will complete the Introductory Forensic Biology/DNA Unit Training Checklist (LAB-DNA-TM-01).

03 BIOLOGICAL EVIDENCE SCREENING UNIT

DNA-TM-03-01 BLOOD SCREENING

Purpose Familiarize the trainee with the procedures for screening evidence for the presence and presumptive testing for blood.

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How recognition of potential bloodstains is essential to the work of a forensic serologist.

B. How the presumptive blood tests are tools for recognizing bloodstains

C. How the results of presumptive blood tests lay the foundation for determining the need for further testing.

1.2 Practical

Following the completion of training, the trainee will be able to:

A. Describe the procedure for conducting presumptive testing in their laboratory.

B. Describe the underlying chemical reaction for each presumptive test.

C. Identify the major factors that ensure the reliability of the presumptive test.

D. Name the major categories of false positive reactions with presumptive blood tests.

E. Describe the role and use of controls in presumptive testing.

F. Describe the high dose hook effect for Hematrace testing.

G. Describe the appearance of blood stains under IR if available in trainee's lab.

H. Describe the advantages and disadvantages of different methods of locating blood stains (visual, luminol, IR).

I. Understand the guidelines for determining sample eligibilities for CODIS entry and how these can affect the decision making process during casework screening.

J. Recognize the characteristics that assist in determining trajectory.

K. Properly collect a portion of a possible bloodstain for testing and storage.

L. Prepare a working solution of the presumptive reagents used in the trainee's lab.

M. Conduct and interpret each presumptive test used in the laboratory.

N. Properly document analyses and results of presumptive testing.

O. Recognize possible blood stains on a variety of substrates using visual screening and IR camera (if used by the laboratory).

1.3 Considerations

A. CrimeLite or equivalent lamps

1. The CrimeLite or equivalent lamps are specially designed for detection of forensic stains, fibers, and fingerprints. Commonly called alternate light sources or forensic light sources, these lamps provide intense light of specific wavelengths.
2. Infrared light is invisible, but with forensic light sources which operate outside the visual spectrum, the visual contrast when examining certain types of surface may be enhanced.
3. Most dried bloodstains on cloth are detectable visually because their color, reddish-brown, is different from that of the material on which the bloodstain has been left. On many substrates, however, bloodstains are not readily visible. Under infrared (IR, 800-900nm), bloodstains left on dark backgrounds may be detected.

B. TMB/PHT/LMG Tests

1. The TMB, PHT, and LMG blood tests take advantage of the oxidative properties of the heme group in blood to produce a color change reaction.
2. The PHT test is the most specific test and produces the least amount of false positive reactions.
3. The TMB test is the most sensitive, but has more false positive reactions with chemical oxidants, plant peroxidase, and catalysts.
4. In order to control false positive reactions, TMB is a two-step test, so if a color change occurs prior to the addition of hydrogen peroxide; the test is uninterpretable and will indicate a false positive reaction.
5. Contact with light and air can cause the test to oxidize and produce a color change even in the absence of blood, so a reaction must be interpreted almost immediately after application.

C. Luminol

1. Luminol also uses the oxidative property of heme found in blood.
2. Since luminol is applied directly to the surface, it can cause the stain to become dilute and should be used to detect latent or invisible blood stains.
3. Luminol is also very sensitive and should be retested before reporting.

D. Hematrace

1. Hematrace is used as a human origin test and will use an antigen-antibody reaction against hemoglobin.
2. A “high dose hook effect” can occur causing a false negative to occur when there is too much hemoglobin present in the sample.
3. Hematrace is also sensitive, so it can detect trace levels of hemoglobin found in other body fluids.
4. It will also produce positive results with primate blood and blood from the domestic ferret.

2 Training Outline

2.1 Lesson Plan

A. Blood screening

1. Blood
   - *a) Composition and function of blood and blood components*
   - *b) Properties of hemoglobin*
   - *c) Blood in criminal evidence*
     - *i. Purpose for identification*
     - *ii. Legal aspects*
     - *iii. Limitations*
2. Location and appearance of bloodstains on evidence
   - *a) Visual examination*
     - *i. Oblique lighting*
     - *ii. Stereoscope*
   - *b) Light sources*
     - *i. Infrared Camera (if available in trainee's lab)*
       - ***Appearance***
       - ***Limitations***
     - *ii. Alternate light source*
       - ***Appearance***
       - ***Limitations***
   - *c) Basic bloodstain pattern interpretation*
     - *i. Patterns*
     - *ii. Documentation*
     - *iii. Limitations*
   - *d) Effects of degradation and aging on blood and bloodstains*
3. Collection and preservation
4. Presumptive testing
   - *a) History and general theory of the testing procedures*
   - *b) Tests – use, chemical basis, sensitivity, stability, and specificity*
     - *i. Tetramethylbenzidine (TMB)*
     - *ii. Phenolphthalin (PHT)*
     - *iii. Leucomalachite green (LMG)*
     - *iv. Luminol*

c) *False positives*
   i. *Chemical oxidants*
   ii. *Catalysts*
   iii. *Plant peroxidases*
d) *Controls – use and testing of reagent controls*

5. Confirmatory testing, crystal tests

B. Species origin determination

1. Immunology
   a) *Antigen and antibody structure and function*
   b) *Antigen-antibody reactions*
   c) *Precipitin testing*
      i. *Methods of determination*
      ii. *Cross-reactivity*
   d) *Sensitivity, specificity, and stability of the reagents*
2. HemaTrace
   a) *Conducting the test*
   b) *Advantages and disadvantages*

C. Blood screening in casework

1. Case documentation
2. Trace evidence collection
3. Location, identification, and documentation of bloodstains
4. Documentation of species origin testing
5. Collection and preservation of evidentiary and reference samples
   a) *Cutting*
   b) *Swabbing*
   c) *Trace DNA*
   d) *Minitapes*
6. Counting examinations
7. Reporting statements
8. Discussion of guidelines for sample entry into CODIS

2.2 Required Readings

Readings are selected based on the presumptive blood test methods for which the trainee seeks qualification. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   1. Approved Standard Abbreviations List;
   2. Facilities;
   3. Analytical Controls;
   4. Case Documentation;
   5. Evidence Handling;
   6. Physical Evidence Examination;
   7. Physical Evidence Collection;
   8. Capital Offense Case Processing Guidelines;
   9. Presumptive Blood Tests;
   10. Report Writing Guidelines;
   11. Guidelines for Counting Examinations;
   12. Commercial Reagents.

B. Bevel, and Gardner. Bloodstain Pattern Analysis with an Introduction to Crime Scene Reconstruction 3rd edition. 2008, pp. 1-131, 165-170, 261-274.

   (may be met by attending a blood spatter analysis class that has been approved by the Technical Leader prior to casework or by completion of the reading)

C. Bevel T. Violent crime scene reconstruction using bloodstain interpretation (electronic presentation). Forensic Education Specialties, Inc. 1984, Parts 1-3.

   (may be met by attending a blood spatter analysis class that has been approved by the Technical Leader prior to casework or by viewing the electronic presentation)

D. Olsen R. Sensitivity comparison of blood enhancement techniques. Identification News. August, 1986, pp. 5-8.

E. Cox M. Effect of fabric washing on the presumptive identification of bloodstains. J For Sci. 1990. 35(6), pp. 1335-1341.

F. Ponce AC, and Verdú Pascual FA. Critical revision of presumptive tests for bloodstains. For Sci Comm. 1999. 1(2).

G. Scientific Working Group on Materials Analysis. Trace Evidence Recovery Guidelines. January 1998. For Sci Comm. October 1999. 1(3).

H. NDIS Forensic Index Decision Tree. FBI. (most recent version).

I. Lee H. Identification and Grouping of Bloodstains. Saferstein R, ed. Forensic Science Handbook, Volume I. Prentice-Hall, Englewood Cliffs, New Jersey, 1982, Chapter 7, pp 272 – 276.

J. Local policies/validations as applicable.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the method described in the reading.

A. TMB

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Tetramethylbenzidine Solution (DNA-09-22)
2. Garner DD, et al. An evaluation of tetramethylbenzidine as a presumptive test for blood. J For Sci. 1976. 21(4), pp. 816-821.
3. Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Department of Justice, National Institute of Justice. 1983. p 101 (introduction), Section 6.6.10 and Section 6.8.
4. Safety Data Sheet for 3,3,5,5-Tetramethylbenzidine

B. Luminol

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Luminol (DNA-04-03)
2. Lytle, and Hedgecock. Chemiluminescence in the visualization of forensic bloodstains. J For Sci. 1978. 23(3), pp. 550-562.
3. Laux, D. Effects of luminol on the subsequent analysis of bloodstains. J For Sci. 1991. 36(5), pp. 1512-1520.
4. Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Department of Justice, National Institute of Justice. 1983. p 101 (introduction), Section 6.7 and Section 6.8.
5. Safety Data Sheet for luminol

C. Hematrace

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Species Origin Test – Hematrace (DNA-04-06)
2. Abacus Diagnostics. ABAcard HemaTrace For the Forensic Identification of Human Blood. Technical Information Sheet. Abacus Diagnostics. (most recent version).

D. Leucomalachite Green

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Leucomalachite Green Solution (DNA-09-11)
2. Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Department of Justice, National Institute of Justice. 1983. p 101 (introduction), Section 6.5 and Section 6.8.
3. Safety Data Sheet for Leucomalachite green

E. Phenolphthalin

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Phenolphthalin Solution (DNA-09-13)

2. Gaensslen RE. Sourcebook in Forensic Serology, Immunology, and Biochemistry. US Department of Justice, National Institute of Justice. 1983. p 101 (introduction), Section 6.3 and Section 6.8.
3. Safety Data Sheets for Phenolphthalin

F. IR

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Infrared Camera (DNA-04-01A)
2. Lee, W., Khoo, B. Forensic Light Sources for Detection of Biological Evidences in Crime Scene Investigation: Review. Malaysian Journal of Forensic Sciences. 1. 2010. pp.17-27.
3. Local validation for IR.
4. Any local policies as applicable.
5. Schotman et al. Understanding the Visibility of Blood on Dark Surfaces: A practical evaluation of visible light, NIR, and SWIR images. Forensic Science International. 257. 2015. pp 214-219.

3 Practice

3.1 Safety

A. Gloves must be worn during reagent preparation and testing. Clothing may protect unbroken skin; broken skin should be covered. Eye protection is recommended during reagent preparation and handling of liquid body fluids. Chemicals used may be carcinogenic or caustic. Blood may contain infective agents. Use universal precautions during evidence handling. If a biological spill were to occur, refer to the most recent version of the Safety Manual, Biological Spills .

B. Zinc, used in the preparation of leucomalachite green and phenophthalin solutions, may burn on exposure to oxygen. Follow preparation instructions and warnings carefully. Always wear appropriate eye protection while using the alternate light source and avoid looking directly into the light. Follow safety and use directions provided with the instrument.

3.2 Standards, Controls, and Reagent Preparation

A. Standards and Controls

1. On the day of use, test blood presumptive reagents using a positive and negative control. Record results on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent.
2. Prior to first use, each new lot of ABAcard Hematrace test kits must be tested according to HemaTrace Reagent Quality Control form (LAB-DNA-09).
3. The lot number and expiration date of all reagents used for testing must be documented in the case record.

B. Reagent Preparation

1. Blood presumptive reagents: Tetramethylbenzidine, phenolphthalin, leucomalachite green, and luminol as available.
2. Refer to the DNA SOP, Reagents section

3.3 Equipment

- Balances (0.02-25 g)
- Pipettes
- Stirring hot plate
- Infrared Camera
- A compilation of training samples consisting of some or all of the following:
  - Known human blood stains of varying concentrations (neat, 1:2, 1:4, 1:8, and 1:16, buccal swabs, semen stain (1:4 dilution), animal bloodstain(s)
  - Unknown and/or mixed stains on different fabrics
  - Different sources of environmental chemicals and/or plant substances (including substances that have a blood-like appearance), for example: ketchup, tomato juice, rust, copper, lemon, orange, banana, flour, horse radish, onion, or turnip

3.4 Observed Performance

The trainer, or their designee, must be present to demonstrate and observe each technique as well as to discuss findings, documentation, and safety issues.

A. Tetramethylbenzidine (TMB) presumptive test
   1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
   2. Trainee will observe the trainer demonstrate the TMB presumptive test.

B. Phenolphthalin (PHT) presumptive test
   1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
   2. Trainee will observe the trainer demonstrate the PHT presumptive test.

C. Leucomalachite green (LMG) presumptive test
   1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
   2. Trainee will observe the trainer demonstrate the LMG presumptive test.

D. Luminol presumptive test
   1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
   2. Trainee will observe the trainer demonstrate the Luminol presumptive test.

E. HemaTrace species origin determination
   1. Trainer and trainee will discuss safety, interpretation, and limitations of the test.
   2. Trainee will observe the trainer demonstrate the HemaTrace species origin test.

F. Infrared Light Source
   1. Trainer and trainee will discuss safety, interpretation and limitations of the infrared light source.
   2. Trainee will observe the trainer demonstrate the infrared light source.

G. Blood evidence processing: Trainee will observe the analysis of at least five typical blood cases including casework documentation, collection of trace evidence, preservation of reference blood and saliva standards, analysis of evidence for the presence of blood, and the collection and preservation of evidentiary stains.

H. CODIS

1. Trainer will discuss the legal basis of CODIS, as well as the indices regularly used by the lab. Discussion should focus on what types of samples may or may not be allowed in the CODIS database for typical blood evidence cases. The trainer may use actual cases to demonstrate CODIS entry decision-making. The trainer may access the CODIS Computer Based Training courses to demonstrate further scenarios.
2. Trainer will demonstrate the use of the NDIS Forensic Index Decision Tree.

3.5 Supervised Performance

A. Tetramethylbenzidine (TMB) presumptive test

1. Trainee will prepare the TMB reagent.
2. Trainee will test a portion of each of the training samples with the TMB reagent.

B. Phenolphthalin (PHT) presumptive test

1. Trainee will prepare the PHT reagent (unless purchased kit is available).
2. Trainee will test a portion of each of the training samples with the PHT reagent.

C. Leucomalachite green (LMG) presumptive test

1. Trainee will prepare the LMG reagent.
2. Trainee will test a portion of each of the training samples with the LMG reagent.

D. Luminol presumptive test

1. Trainee will prepare the luminol reagent.
2. Trainee will test a portion of selected training samples with the luminol reagent.

E. HemaTrace species origin determination

1. Trainee will test the following for human origin: stains of blood diluted 1:2, 1:4, 1:8, and 1:16 in dIH2O.
2. Trainee will test the following for human origin: non-human animal bloodstain extract, human stain or swab extracts (e.g., blood, buccal, semen, vaginal, urine).

F. Infrared Light Source

Trainee will view a portion of selected training samples with the infrared light source.

3.6 Independent Exercises

As practice, trainee will independently screen a compilation of at least 5 training samples using the blood screening tests for which qualification is sought. The training samples should include some or all of the following: known bloodstains of varying concentrations, animal bloodstains, sources of environmental chemicals and/or plant substances, and other substances that have a blood-like appearance on dark material. Trainee may demonstrate casework documentation and collection and preservation of evidentiary stains using these samples.

4 Assessment

4.1 Competency and Qualifying Examination

A. Trainee will test a minimum of five competency samples using each presumptive blood test for which they seek qualification (e.g. TMB, PHT, LMG, Luminol, Hematrace, IR). The same five samples may be used for all tests.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent forensic biology analysis (blood screening).

4.2 Evaluation of Training

The trainee and trainer will complete the Biological Evidence Screening Training Checklist (LAB-DNA-TM-02).

DNA-TM-03-02 SEMEN IDENTIFICATION AND DETERMINATION

Purpose Familiarize the trainee with the procedures for screening evidence for the presence of semen, presumptive tests, and confirmatory tests.

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The identification of semen and semen stains and their importance in criminal offenses such as homicides and sexual assaults.

B. How the presence of semen on the body of a victim or an item of evidence can provide a direct link to a potential suspect as well as potentially provide substantiation of the sexual nature of the perpetrator's contact.

C. How information about the presence, location, and state of degradation of semen can possibly help corroborate a victim's statement or provide a basis for further investigation.

D. How, unlike blood testing in our laboratory, presumptive tests for semen can generally be confirmed through further testing.

1.2 Practical

A. Upon completion of training for the screening of evidence containing semen, the trainee will be able to:

1. Describe methods of locating semen stains on various substrates.
2. Properly collect a portion of a possible semen stain for testing and storage.
3. Describe the advantages and disadvantages of different methods of locating semen stains.
4. Describe the appearance of various stains under ultraviolet (or alternate or intense) light.
5. List the typical components of a sexual assault kit.
6. Understand the guidelines for determining sample eligibility for CODIS entry and how these can affect the decision making process during casework screening.
7. Properly document analyses and results.

B. Upon completion of training for the semen presumptive tests, the trainee will be able to:

1. Prepare a working solution of the acid phosphatase test reagent.
2. Prepare working solutions of the nuclear fast red and picroindigocarmine stain reagents or describe the general procedure and underlying principle.
3. Extract semen stains from a variety of substrates.
4. Conduct and interpret each semen presumptive test used in the laboratory.
5. Describe the underlying chemical reaction for the acid phosphatase test and p30 test.
6. Describe and explain the purpose of acid phosphatase mapping.

7. Identify the major factors that affect the reliability of the acid phosphatase test.
8. Name the major categories of false positive reactions with the acid phosphatase test.
9. Describe the role and use of control samples for the acid phosphatase test and p30 test.
10. List the advantages and limitations of the p30 test.
11. Identify the major factors that affect the reliability of the p30 test.
12. Explain under what circumstances a false positive reaction may occur with the p30 test.
13. Describe the high dose hook effect.
14. Explain the need for confirmatory testing.

C. Upon completion of training for microscopic identification of spermatozoa, the trainee will be able to:

1. Diagram, color, and identify each part of a normal stained spermatozoon.
2. Diagram, color, and identify each part of a normal stained epithelial cell.
3. Describe alternative methods (and their limitations) for recovering spermatozoa from a stained substrate.
4. Name and describe the procedure for staining a microscope slide for identification of spermatozoa.
5. Demonstrate methods of preparing a microscope slide from a semen stain.
6. Conduct a microscopic examination for spermatozoa.
7. Use the guidelines governing CODIS entry to aid in determining the location, type, and number of samples to collect.

1.3 Considerations

A. Alternate Light Source (ALS)

1. Direct, intense light will degrade DNA over time. Limit exposure of stains to ALS light.
2. Fluorescence of semen stains is thought to be due to the conversion of non-protein substances into compounds capable of fluorescence, the presence of flavins (an organic compound) in semen, as well as the growth of the fluorescent bacterium *Pseudomonas fluorescens* in the stain. Therefore, older stains may fluoresce more efficiently than newer stains.
3. Urine, saliva, nasal secretions, sweat, and some food stains are also capable of fluorescence, though it is usually less intense than semen stains.

B. Acid Phosphatase (AP)

1. Acid phosphatase activity has been detected in samples collected for up to 7 days postmortem in vaginal samples, for up to 36 hours in the oral cavity, and for up to 24 hours in the rectum, depending on body temperature. Seminal acid phosphatase activity in the vagina is greatly reduced after 24 hours in live victims.

2. Semen stains can lose up to half of their acid phosphatase activity on drying if not dried quickly and kept dry. The activity continues to decline even in cold storage (about 50-80% decline over 5 months at room temperature in one study), though it has been detected in stains up to 6 years old.
3. Heating a sample to 60°C for 5 minutes or 37°C for 14 days destroys acid phosphatase activity; and a sample kept at room temperature for a month shows 30% reduction in activity.
4. A few chemicals that could be found as components of vaginal deodorants or spermicidal contraceptives can inhibit acid phosphatase or produce a false positive reaction.
5. It is possible for an AP test to be negative even when sperm are present.
6. Only the prostatic secretions of humans and monkeys contain large acid phosphatase concentrations. The level of acid phosphatase in the blood of men with prostatic cancer increases dramatically.
7. Significant levels of acid phosphatase are also found in the juice or extracts of cauliflower, some gourds, gorse, some legume seeds, ginger, okra, onion, and radish.
8. A positive result on an AP spot test may be due to acid phosphatase from another body fluid.
9. Contact with light and air will eventually cause the AP Test Reagent to turn purple even in the absence of acid phosphatase; therefore the reaction must be interpreted within 60 seconds of application of the AP Test Reagent.
10. Stains on evidence may not be homogenous; therefore, the testing activity may vary based on the area of the stain that is tested. For example, the acid phosphatase activity of stains may be more concentrated in the periphery of a stain, whereas the sperm cells may concentrate more in the center.

C. p30 / Prostate Specific Antigen

1. Seminal stains up to a year old can react with anti-p30.
2. Dry semen stains stored at -20°C, 4°C, or room temperature show little loss of p30 over 5 months.
3. P30 may be detected in the vagina up to two days post-coitus.
   a) *The false negative "high dose hook effect" occurs when the p30 concentration in the sample is so high that only some of the p30 molecules are bound by mobile antibodies and the remaining p30 migrates to the area of immobilized antibodies. The free p30 will then bind to the immobilized antibody and prevent it from binding with the mobile antibody-antigen complex, which is necessary for visualization of the dye.*
   b) *The p30 ABAcard is not expected to detect a semen dilution below 1:1,000,000.*
   c) *A dilution of 1:809,200 was barely detectable during validation.*
4. A positive result may be due to p30 from another body fluid.
   a) *Although p30 is normally found only in seminal fluid, p30 is also detectable in the blood of men with prostatic carcinoma and in male urine, probably as a result of drainage from the prostatic ducts into the urethra.*

b) It has been reported in non-prostatic sources such as normal and abnormal breast tissue, various breast fluids (milk, nipple aspirate, cyst fluid), amniotic fluid, and female serum.

c) Vasectomy does not affect the amount of p30 secreted into the seminal fluid.

d) Orangutan and macaque semen contain p30 at concentrations within the human range but have relatively very low acid phosphatase activity.

D. Spermatozoa

1. Many studies have been performed concerning the survival time of sperm in the orifices of both living and non-living victims. Differences in how the information for these studies is collected contribute to differences in reported persistence of sperm cells in the body.

   a) Some studies use volunteer subjects and sample collection that is done at set, controlled time points.

   b) Other studies use data collected from actual cases, and the time since intercourse is based on what is reported by the victim during the forensic interview.

   c) Additionally, some studies report the single longest time since intercourse at which sperm were observed while other studies report an average based on multiple observations.

   d) Finally, sample sets for these studies vary in size.

2. In addition to differences in how persistence studies are conducted, survival time of sperm in the body is affected by the victim's gender, age, activity after sexual contact, time since collection of evidence, and the circumstances surrounding the deposit of semen (e.g. condom use, location of semen deposit, biology of semen donor).

   These differences contribute to the perceived inconsistency of results in the scientific literature concerning the survival time of sperm in the body.

3. In a living victim, the chance of finding sperm on orifice swabs decreases as the time since intercourse increases. The following estimates are for living victims and are based on averages as reported in scientific literature:

   a) Vaginal swabs: the chance of finding sperm is highest within 48 hours of intercourse, and the chance of finding sperm is considered extremely low beyond 96 hours after intercourse.

   b) Anal swabs: the chance of finding sperm is highest within 48 hours of intercourse, and the chance of finding sperm is considered extremely low beyond 48 hours after intercourse.

   c) Oral swabs: the chance of finding sperm is very low in general; however, there is a chance of finding sperm within 15 hours of intercourse.

4. Current Texas Evidence Collection Protocol advises that use of the evidence collection kit may not be necessary if the assault occurred over 120 hours prior to the examination.

5. Semen on rectal or anal swabs may be due to drainage from the vagina.

6. Semen of normal, fertile men can contain almost 40% abnormal forms of spermatozoa. These are generally characterized by elongated, thin, or irregular head shapes or bent or coiled tails.
7. Fibers and certain bacteria and molds can resemble sperm tails. Yeast or various other cells may resemble sperm heads. Identification of sperm should be based on observation of intact cells if at all possible.

2 Training Outline

2.1 Lesson Plan

A. Biological aspects of the male and female reproductive systems
   1. Vaginal fluid
      a) Biochemistry and function
      b) Chemical and cellular components
   2. Semen
      a) Biochemistry and function
      b) Chemical and cellular components
      c) Persistence of semen components
         i. in vaginal, anal, and oral cavities
         ii. in stains

B. Semen in criminal evidence
   1. Purpose of identification
   2. Legal aspects
   3. Limitations

C. Semen screening
   1. Locating semen stains
      a) Visual examination
         i. appearance
         ii. limitations
      b) Light sources
         i. appearance
         ii. limitations
   2. Collection and preservation
   3. Presumptive testing
      a) History and general theory of the testing procedures
      b) Tests – use, chemical basis, sensitivity, stability, and specificity
         i. acid phosphatase – spot test and mapping

ii. choline and spermine

iii. Identification of p30 - ABAcard

c) Quality control

i. False positives

ii. Reagent controls – use and interpretation

iii. Substrate controls – use and collection

4. Confirmatory tests

a) Sample extraction methods

b) Identification of spermatozoa

i. microscopy review

ii. staining techniques

iii. sperm morphology

iv. morphology of other common components of smear slides

5. Semen screening in casework

a) Sexual assault evidence

i. sexual assault kits

ii. clothing, bedding, miscellaneous evidence

b) Case documentation

c) Trace evidence collection

d) Location, identification, and documentation of semen evidence

e) Collection and preservation of evidentiary and reference samples

i. Cutting

ii. Swabbing

f) Reporting statements

g) Guidelines for sample entry into CODIS

2.2 Required Readings

Readings completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Comprehensive:

1. Baechtel SF. The identification and individualization of semen stains. Saferstein R, ed. Forensic Science Handbook, Volume II. Prentice-Hall, Englewood Cliffs, New Jersey. 1988. Chapter 7, pp. 347-369.
2. Greenfield A, and Sloan MM. Identification of biological fluids and stains. James SH and Nordby JJ, eds. Forensic science: An introduction to Scientific and Investigative Techniques. 2nd. Ed. CRC Press. 2005. Chapter 14, pp. 261-269.
3. Saferstein R. Criminalistics An Introduction to Forensic Science, 7th edition. Pearson Prentice Hall. 2004. pp. 342-350.

4. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Facilities;*
   b) *Analytical Controls;*
   c) *Case Documentation;*
   d) *Evidence Handling;*
   e) *Physical Evidence Examination;*
   f) *Physical Evidence Collection;*
   g) *Capital Offense Case Processing Guidelines;*
   h) *Alternate Light Source;*
   i) *Presumptive Semen Test – Acid Phosphatase;*
   j) *Body Fluid Stain Extraction;*
   k) *Spermatozoa Examination;*
   l) *P30 Identification;*
   m) *Report Writing Guidelines;*
   n) *Guidelines for Counting Examinations;*
   o) *AP Test Reagent;*
   p) *Nuclear Fast Red Solution;*
   q) *Picroindigocarmine;*
   r) *Commercial Reagents.*
5. Any local policies/validations as applicable.

B. Presumptive Testing:

1. Schiff AF. Reliability of the acid phosphatase test for the identification of seminal fluid. J For Sci. 1978. 23(4).
2. Banerjee PK, and Bhattacherjee TK. Detection of spermine, choline, and acid phosphatase in the vegetable. J Police Sci and Admin. 1975. 3(3), pp. 280-283.
3. McCloskey KL, Muscillo GC, and Noordewier B. Prostatic acid phosphatase activity in the postcoital vagina. J For Sci. 1975. 20(4), pp. 630-636.

C. Identification of Spermatozoa:

1. Payne RL. Cytology for the forensic scientist. Unpublished. 1988.
2. Willott GM. Frequency of azoospermia. For Sci Int. 1982. 20, pp. 9-10.

D. Identification of p30:

1. Abacus Diagnostics. ABAcard p30 Test for the Forensic Identification of Semen. Technical information sheet. Abacus Diagnostics,. (most recent version).
2. Benton K, Donahue J, and Valadez M. Analysis of the OneStep ABAcard PSA test for use in the forensic laboratory. Texas Department of Public Safety Crime Laboratory Service. Unpublished. 1998, pp. 1-8.
3. Yu H. Clinical implications of prostate-specific antigen in men and women. J Gender-Specific Med. 2000. 3(2), pp. 45-48, 53.

E. Semen Screening in Casework:

1. The Office of the Attorney General. Sexual Assault Prevention and Crisis Services Division. Texas Evidence Collection Protocol. 1998. pp. 21-42.
2. Casey D, et al. The Persistence of Sperm and the Development of Time Since Intercourse (TSI) Guidelines in Sexual Assault Cases at Forensic Science Ireland, Dublin, Ireland. J For Sci. 2017. 62(3), pp. 585-592.

F. Safety:

1. National Institute for Occupational Safety and Health (NIOSH). Health hazard alert – benzidine-, 0-tolidine-, and o-dianisidine- based dyes. Department of Health and Human Services Publication number 81-106. 1980. pp. 1-7.
2. Safety Data Sheets:
   - α-naphthyl acid phosphate, calcium salt
   - acetic acid (glacial)
   - Aerosol 22
   - aluminum sulfate
   - ethanol, absolute
   - indigocarmine
   - nuclear fast red
   - o-dianisidine, tetrazotized
   - phosphate buffered saline solution
   - picric acid or saturated picric acid solution
   - sodium acetate, trihydrate
   - sodium chloride
   - xylenes, mixed

G. CODIS: NDIS Forensic Index Decision Tree. FBI. (most recent version)

3 Practice

3.1 Safety

A. Gloves must be worn during reagent preparation and testing. Clothing may protect unbroken skin; broken skin should be covered. Eye protection is recommended during reagent preparation and handling of liquid body fluids. Chemicals used may be carcinogenic or caustic. Blood may contain infective agents. Use universal precautions during evidence handling.

B. Picric acid, used in the preparation of picroindigocarmine stain, is explosive when dry. Follow storage and preparation instructions and warnings carefully.

C. Xylenes can cause irritation when inhaled.

D. α-naphthyl acid phosphate and o-dianisidine are suspected carcinogens. Application of the AP spot test reagent using a spray bottle must be performed in a chemical fume hood.

E. Always wear appropriate eye protection while using the alternate light source and avoid looking directly into the light. Follow safety and use directions provided with the instrument.

3.2 Standards, Controls, and Reagent Preparation

A. Standards and Controls

1. On each day of use, the acid phosphatase spot test solution reagent must be tested using a positive and negative control and the results recorded on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent.
2. Prior to first use, each new lot of ABAcard p30 test kits must be tested according to HemaTrace Reagent Quality Control form (LAB-DNA-09).
3. The lot number and the expiration date of all reagents used in testing must be included in the case record.

B. Reagent Preparation

1. Acid phosphatase test(s)
2. Christmas tree stain: nuclear fast red solution, picroindigocarmine solution (unless pre-made reagents are purchased)

3.3 Equipment

- Oven
- Alternate light source
- Balance/scale
- Hot plate or microwave oven
- Centrifuge
- Microscope
- Refrigerator
- Sonicator
- Stir Plate
- Pipettes
- Vortex
- A compilation of training samples consisting of some or all of the following based on the types of evidence routinely submitted to the trainee's laboratory:
  - Known semen stains of varying concentrations, bloodstains, vaginal fluid stains, urine stains, sweat stains, milk stains, vaginal deodorant, oral rinses, and spermicide.
  - Unknown and/or mixed stains on different fabrics.
  - Different sources of environmental chemicals and/or plant substances, for example: cucumber, breast milk, almond, cauliflower, Brussel sprouts, garlic, turnip, raisins, mango, ginger, or dates.

3.4 Observed Performance

The trainee must be trained to perform each test which is used in their respective casework laboratory. The trainer must be present to demonstrate each technique as well as to discuss findings, documentation, and safety issues.

A. Location of semen stains

1. Trainer and trainee will discuss the appearance of a variety of body fluid stains on various substrates.
2. Trainer and trainee will discuss safety, interpretation and limitations of using an alternate light source.
3. Trainer will demonstrate the use of an alternate light source.

B. Acid phosphatase (AP) spot test

1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
2. Trainer will demonstrate the use of the acid phosphatase test.

C. Acid phosphatase (AP) mapping

1. Trainer and trainee will discuss safety, interpretation and limitations of the test.
2. Trainer will demonstrate the use of the acid phosphatase test for mapping.

D. Stain Extraction

1. Trainer and trainee will discuss appropriate use and limitations of each extraction technique.
2. Trainer will demonstrate stain extraction techniques.

E. ABAcard test for p30

1. Trainer and trainee will discuss limitations of the test, including potential false negative reactions.
2. Trainer will demonstrate the use of the ABAcard test for p30.

F. Identification of Spermatozoa

1. Trainer and trainee will discuss morphology of various cellular and acellular components in typical microscope slide preparations for sperm identification.
2. Trainer will demonstrate the use of a microscope to identify spermatozoa.

G. Semen screening in casework

Trainee will observe the analysis of at least five typical sexual assault cases including casework documentation, collection of trace evidence, preservation of reference blood and saliva standards, analysis of evidence for the presence of semen, and the collection and preservation of evidentiary stains.

H. CODIS

1. Trainer will discuss the legal basis for CODIS, as well as the indices regularly used by the lab. Discussion should focus on what types of samples may or may not be allowed in the CODIS database for typical sexual assault evidence cases. The trainer should discuss how the presence of a consensual partner may affect CODIS eligibility. The trainer may use actual cases to demonstrate CODIS entry

decision-making. The trainer may access the CODIS Computer Based Training courses to demonstrate further scenarios.

2. Trainer will demonstrate the use of the NDIS Forensic Index Decision Tree.

3.5 Supervised Performance

The trainee must be trained to perform each test which is used in their respective casework laboratory. The trainer must be present to observe each technique as well as to discuss findings, documentation, and safety issues.

A. Location of semen stains

Trainer will observe trainee's use of an alternate light source.

B. Acid phosphatase (AP) spot test

1. Trainee will prepare the AP reagent(s).
2. Trainee will test each of the training samples with the AP reagent(s).

C. Acid phosphatase (AP) mapping

Trainee will test selected training samples with the AP mapping test.

D. Stain extraction

1. Trainee will extract a portion of selected training samples.
2. Trainee will use each available technique at least once.

E. ABAcard test for p30

Trainee will test the extracted training samples using the ABAcard test.

F. Identification of spermatozoa

1. Trainee will prepare the nuclear fast red and picroindigocarmine stains.
2. Trainee will prepare and stain the training smears.
3. Trainee will examine the stained training smears using normal light microscopy at the full range of magnification available.

G. Semen screening in casework

Under direct observation by the trainer, trainee will demonstrate analysis of semen evidence typical in casework including casework documentation, collection of trace evidence, preservation of standards, analysis for the presence of semen, and collection and preservation of evidentiary stains. This requirement may be met either through practice samples or the mock case required in the overview of the training manual for the Biological Evidence Screening Unit.

3.6 Independent Exercises

As practice, trainee will independently screen a compilation of at least 5 training samples using the semen screening tests for which qualification is sought. The training samples should include some or all of the following: known semen stains of varying concentrations, vaginal fluid stains, urine stains, sweat stains, milk stains, vaginal deodorant, oral rinses, and spermicide, sources of environmental chemicals and/or plant substances, and mixed stains on different fabrics. Trainee may demonstrate analysis of semen evidence typical in casework including casework documentation, and collection and preservation of evidentiary stains using these samples.

4 Assessment

4.1 Competency and Qualifying Examination

A. Trainee will test a minimum of five competency samples for the presence of semen using the methods of testing for semen (i. e. ALS, AP, p30, microscopy).

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent forensic biology analysis (semen screening).

4.2 Evaluation of Training

The trainee and trainer will complete the Biological Evidence Screening Training Checklist (LAB-DNA-TM-02).

DNA-TM-03-03 FORENSIC BIOLOGY REPORT WRITING AND TECHNICAL REVIEW

Purpose Provide trainee with guidelines for biological screening casework report writing and for technical and administrative case reviews.

Prerequisite Introductory Forensic Biology/DNA Unit, Blood Screening and/or Semen Identification and Determination (this module may be completed concurrently with Blood Screening and/or Semen Identification and Determination modules)

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How the laboratory report is used to communicate to its reader both the analytical results and the conclusions of the analyst, conveying the essence of what would be said if the analyst were asked to give an opinion in court.

B. How the report is the method through which the analyst is able to state what the evidence means and also what it does not mean. (It is highly desirable that the report be able to "stand alone" since decisions may be made by police officers, attorneys and the courts, based on the report, with no contact with the analyst.)

C. How a thorough review of each case record is performed to ensure that conclusions and supporting data are reasonable and within the constraints of scientific knowledge.

D. The two separate review components to the case review process.

1. Technical Review

   a) How to conduct an evaluation of documentation to check for consistency, accuracy, and completeness.

   b) How to conduct an evaluation of reports, notes, data and other documents to ensure an appropriate and sufficient basis for the scientific conclusions.

   c) The requirements for personnel who perform technical review (must be a second qualified analyst.)

2. Administrative Review

   a) How to conduct a review of the report and supporting documentation to check for consistency with laboratory policies and for editorial correctness.

   b) The requirements for personnel who perform administrative review (must be conducted by someone other than the analyst completing the case.)

1.2 Practical

Following the completion of training the trainee will be able to:

A. Reduce technical data to a concise conclusion.

B. Write clear, concise reports that will be provided to the submitting officer and prosecuting and defense attorneys.

C. Understand the purpose of case review.

D. Understand the requirements of the technical review and administrative review.

E. Technically review biological screening cases from the testing performed to the written report.

2 Training Outline

2.1 Lesson Plan

A. Report writing

1. General case information
   - *a) Case documentation*
   - *b) Documentation, technical notes, case notes*
   - *c) Policy*
   - *d) Case-related correspondence*
   - *e) Records*
   - *f) Laboratory Reports*
   - *g) Elements in reports*
   - *h) Format of reports*
2. Submission information – Contains information about the evidence containers, date and method of submission, and relevant tracking information. Additional submissions must be clearly marked.
3. Requested analysis – Contains a general statement pertaining to the requested analysis, which can be a mixture of the laboratory's normally used statement and any special requests made by the submitting agency. Include a reference to relevant previous reports or reports previously issued in the same discipline. Also, information regarding evidence transfer between laboratories or resubmissions may be included here.
4. Evidence Description, Results of Analysis and Interpretation
   - *a) Listing of items analyzed for forensic biological analysis*
   - *b) Blood examinations*
   - *c) Semen examinations*
   - *d) Other examinations*
   - *e) Items that were not examined*
5. Investigative leads – This section is directed to the investigating officer to inform them of what additional evidence needs to be submitted in order for DNA analysis or other comparisons to be performed.
6. Disposition – Specific disposition notes should be used to inform the officer if any further action needs to be taken on their part regarding the evidence submitted. It is one way to identify items being retained in the laboratory. This section may also be used to indicate if evidence was forwarded to another lab or section.
7. Other types of reports
   - *a) Supplemental reports*
   - *b) Amended reports*
   - *c) Closed without Analysis reports*

B. Technical Review

1. The technical review will be performed on the final draft. The supporting case information should be organized in a logical fashion with case items highlighted on relevant forms.
2. The results, interpretations, opinions, and conclusions must be reasonable, within the constraints of validated scientific knowledge, and supported by the examination records.
3. The reporting statements should be written to ensure that results are communicated properly and clearly.
4. Refer to the Review of Laboratory Records chapter in the Crime Laoboratory Service Manual for the requirements of the technical review.

C. Administrative review

1. The administrative review will be performed on the final draft to ensure that the reported results are logical and accurate and there are no spelling or grammatical errors.
2. The chain of custody and disposition of the evidence will be reviewed for all evidence associated to the report.
3. Refer to the Review of Records chapter in the Crime Laboratory Service Manual for the requirements of the administrative review.

D. Examination Counting

2.2 Required Readings

Readings are selected based on if the trainee seeks qualification in report writing, technical review, or both.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Report Writing

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   - *a) Review of Laboratory Records;*
   - *b) Document Management and Deviations;*
   - *c) Quality Incident (QI) and Action Plan Process (QAP);*
   - *d) Preventive Actions;*
   - *e) Laboratory Records;*
   - *f) Laboratory Reports, Letters, and Certificates;*
   - *g) Expunction and Destruction of Laboratory Records and Information;*
   - *h) Electronic Storage and Archival of Records;*
   - *i) Records Requests and Release of Laboratory Records and Information;*
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   - *a) Approved Standard Abbreviations List;*
   - *b) Response to Quality Issues;*

c) *Guidelines for Technical Case Review;*
d) *Case Documentation;*
e) *Report Writing Guidelines;*
f) *Guidelines for Counting Examinations.*

3. Federal Bureau of Investigation. Quality Assurance Standards for Forensic DNA Testing Laboratories (most recent version). Standard 11.

B. Technical Review

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Review of Laboratory Records;*
   b) *Document Management and Deviations;*
   c) *Laboratory Records;*
   d) *Laboratory Reports, Letters, and Certificates;*
   e) *Evidence and Database Sample Integrity.*
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Analytical Controls;*
   b) *Guidelines for DNA Technical Review;*
   c) *Case Documentation;*
   d) *Report Writing Guidelines.*
3. Federal Bureau of Investigation. Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (most recent version). Standard 12.
4. LIMS Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *General (chapters within this section);*
   b) *Forensic Biology Workflow.*

3 Practice

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

3.1 Safety

None

3.2 Standards, Controls, and Reagent Preparation

None

3.3 Equipment

None

3.4 Observed Performance

A. For report writing the trainer will provide 10-20 cases involving forensic biological analysis to the trainee to allow familiarity with the method of report writing in the laboratory.

B. For technical review the trainer will demonstrate the technical review of at least one forensic biology case.

3.5 Independent Exercises

A. If qualification is sought in report writing, the trainee must complete the following exercises, which also serve as the competency test in report writing:

1. The trainee will be provided with data (case report removed) from 5 forensic biology cases previously analyzed and reported.

   ***Note**: All case information including case number, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.*

2. Using the DNA SOP and instruction from the trainer, the trainee must interpret the results and prepare a report for each case according to the format in his/her laboratory.

3. Upon completion, the trainee may compare their statements with the actual statements reported on the case and discuss their conclusions/reports with the trainer.

B. If qualification is sought in technical review, the trainee must complete the following exercises, which also serve as the competency test in technical review:

1. The trainer will provide a minimum of 5 biological screening cases on which the trainee will perform a preliminary technical review, prior to the actual case review by a qualified analyst. The set of cases should include a variety of offenses. These reviews shall be evaluated by the trainer, and the trainer will discuss any discrepancies found during the actual case review with the trainee.

   ***Note**: All case information including case number, names, and any other identifying information must not be included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.*

2. After an evaluation of the trainee's preliminary case reviews, the trainer and/or Technical Leader may assign further preliminary technical reviews until several cases have been reviewed by the trainee without significant discrepancies between the actual technical review.

4 Assessment

4.1 Competency and Qualifying Examination

A. The examination will consist of the independent exercises described above. This will serve as the competency test in report writing and case review for forensic biological analysis.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent report writing and technical review for forensic biology analysis.

4.2 Evaluation of Training

The trainee and trainer will complete the Biological Evidence Screening Training Checklist (LAB-DNA-TM-02).

04 MALE SCREENING UNIT

DNA-TM-04-01 MALE SCREENING – SAMPLE PREPARATION

Purpose Provide trainee with guidelines for male screening sample preparation

Prerequisite Introductory Forensic Biology/DNA unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of how male screening is an alternative screening method to traditional serological testing of sexual assault samples.

Note: Analysts who have not completed biological evidence screening training may be trained to prepare samples for the male screening process.

1.2 Practical

Following the completion of training the trainee will be able to:

A. Understand the purpose, benefits, and limitations of the male screening process.

B. Understand the quality control and precautionary measures associated with sample preparation for the male screening process.

C. Understand the required documentation.

2 Training Outline

2.1 Lesson Plan

A. Identification of cases eligible for the method

B. Itemization and documentation of evidence in LIMS

C. Sample Preparation

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. SWGDAM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits in a Laboratory (most recent version)

B. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

1. Case Documentation;
2. Evidence Handling;
3. Physical Evidence Examination;
4. Male Screening Process – Plexor HY (Sections 1 - 4 and 6);

C. LIMS Manual. Texas Department of Public Safety Crime Laboratory (most recent version) – Male Screening Workflow.

D. Any local policies/validations as applicable

3 Practice

3.1 Safety

Gloves must be worn during evidence processing. Clothing may protect unbroken skin; broken skin should be covered. Use universal precautions during evidence handling.

3.2 Standards, Controls, and Reagent Preparation

None

3.3 Equipment

- Scalpel
- Forceps
- Scissors
- Microcentrifuge tubes
- Tube racks

3.4 Observed Performance

A. Trainer or mentor will give instruction on the types of cases eligible for male screening.

B. Trainer or mentor will provide instruction and/or demonstrate the itemization of evidence as required for sample preparation.

C. Trainer or mentor will demonstrate how to prepare samples for testing.

D. Trainer or mentor will demonstrate documentation and sample preparation for samples going forward for DNA analysis.

3.5 Independent Exercises

Practice Set: All analysts being trained in this procedure will receive a practice set of ten samples to be prepared for the male screening process.

4 Assessment

4.1 Competency and Qualifying Examination

A. A competency set of 10 samples will be prepared for the male screening process. These samples must contain biological material.

Note: *The only way to grade the competency set is to use the quantification results obtained from the competency. Therefore, the samples must be processed through the remainder of the male screening process either by the trainee or by another qualified analyst. If the trainee is qualifying for Male Screening Preparation and Male Screening Process concurrently, this requirement can be satisfied by the use of the same 10 samples.*

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent male screening sample preparation of casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Male Screening Training Checklist (LAB-DNA-TM-03).

DNA-TM-04-02 MALE SCREENING PROCESS

Purpose Provide trainee with instruction on performing the male screening process including interpretation

Prerequisite Introductory Forensic Biology/DNA Unit, Male Screening – Sample Preparation module, Liquid Handling Robotics module (abbreviated; unless waived) (Male Screening – Sample Preparation and Liquid Handling Robotics may be completed concurrently with this module).

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The purpose, benefits, and limitations of the male screening process including the Plexor HY DNA quantitation system.

B. The quality control and precautionary measures associated with the male screening process.

C. The theory behind the male screening procedure based on laboratory validation.

D. How validated quantification thresholds are used to determine if further analysis is needed for samples.

1.2 Practical

Following the completion of training the trainee will be able to:

A. Perform the male screening process, if necessary.

B. Properly document analyses and results.

C. Interpret data obtained through the male screening process, if necessary.

D. Apply quantification thresholds to data and determine when further analysis is needed based on these values.

E. Understand the next steps in the casework workflow.

1.3 Considerations

A. Background

1. Male screening uses a DNA approach as an alternative screening method to traditional serological testing of sexual assault samples. Outlined are the training objectives needed to be accomplished in order to become competent in various aspects of the Male Screening process.

2. In one version of this process an abbreviated lysis, or extraction, is performed on the samples. Instead of further purification of the DNA contained in the lysate, the lysate proceeds directly to the quantification step. An alternative version has been validated using purified DNA for the quantification step.

3. During the quantification step, the presence of male DNA is determined through the use of a quantification system that can detect the presence of amplifiable autosomal human (and higher primate) and male-specific DNA. A ratio of amplifiable autosomal human DNA to male-specific DNA is also developed; this

ratio is useful in determining whether or not and in which way (autosomal or Y-STR) to proceed with further sample analysis.

4. The 7500 Real Time PCR System can be used to determine the relative quantity of a target nucleic acid sequence in a sample by analyzing the cycle-to-cycle change in fluorescent signal as a result of amplification during a PCR.

5. The DNA quantification assay combines three 5' nuclease assays which are a target-specific human autosomal assay, a target-specific human male DNA assay and an internal PCR control (IPC) assay. The purpose of the IPC (Internal PCR Control) is to distinguish between true negative sample results and reactions affected by inhibitors, assay setup and chemistry/instrument failures.

6. The autosomal target is labeled with fluorescein (FL), the Y target is labeled with CO560, the IPC is labeled with CR610, and the passive reference is labeled with IC5. The passive reference is used to normalize the reaction and account for well-to-well differences due to sample dispensing.

B. Standard Curve

The standard curve is a graph of the C_q (quantification cycle) of quantification standard reactions plotted against the starting quantity of the standards. The software calculates the regression line by calculating the best fit with the quantification standard data points. Positive amplification occurs when the C_q value for the detector is <38. Because samples contain unknown amounts of DNA, a large range of C_q values is possible.

C. R^2 value

The R^2 value measures the closeness of fit between the standard curve regression line and the individual C_q data points of quantification standard reactions. An R^2 value ≥0.98 indicates a close fit.

D. Slope

The slope of this regression line indicates the PCR amplification efficiency for the assay. Slope values typically fall between -3.2 and -4.0 for the autosomal target and between -3.0 and -3.6 for the Y target. A slope of -3.3 indicates 100% amplification efficiency.

E. Y-intercept

The Y-intercept indicates the expected C_q value for a sample with quantity = 1. Some variation in the Y-intercept value may be observed. These variations may be a result of: pipetting variation during standard setup, lot-to-lot variations in the kits, target-to-target variation, or instrument-to-instrument variation. Minor variations do not affect assay performance or quantification accuracy; however each lab should monitor the Y-intercept over time for each instrument to determine if variations become significant.

F. IPC

Data from samples are compared to data from DNA standards of similar autosomal C_q values. Inhibition is noted if the IPC C_q value of an unknown sample is delayed by more than two cycles. If this occurs the software will flag the sample with "check IPC" and the IPC amplification should be reviewed. If the sample is displaying partial or complete inhibition, this may require the sample to be processed further (with either presumptive testing or DNA analysis) depending on case results.

1. **Negative Results**: No human and/or male DNA is detected when the IPC target amplified but the human-specific target and/or male specific target did not amplify.

2. **Invalid IPC Results**: If the human-specific target and/or male-specific target *and* the IPC target failed to amplify, this is highly indicative of complete PCR inhibition but it is not possible to distinguish between the absence of DNA, PCR reaction failure, and PCR inhibition.

3. **IPC Results Inconclusive**: With extremely high concentrations of human genomic DNA (>10 ng/µL), competition between the human-specific and/or male-specific and IPC PCR reactions appears to suppress IPC amplification for that sample. If the target amplifies with low C_q and high ΔR_n results and the sample gives a reading of ≥0.01ng/µL in the [Y] result, it is unlikely that PCR inhibitors are present and the sample is positive for male DNA. In these cases, appearance of suppression or failure of IPC amplification renders the IPC result inconclusive.

4. **PCR Inhibition**: Weak amplification (high C_q and low ΔR_n value) of the human target and/or male-specific target (sample has a value of <0.01ng/µL in the [Y] result) and no or weak amplification of the IPC may indicate PCR inhibition in the sample.

G. Melting Temperature

The melting temperature (T_m) is empirically determined by plotting the change in fluorescence with temperature versus temperature and determining the temperature at which the greatest rate of change in fluorescence occurs. Melt curves from samples are compared to the melt curve threshold and window in the software. If potential problems with melt curves are noted, the software will flag the sample with “check melts” and the melt curve should be reviewed. Observation of no melt curve may be an indication of poor amplification. Observation of melt curves that are shifted within the window or do not cross the threshold may be an indication of non-specific, non-human amplification. The average expected T_m is 79-81ºC for the autosomal human target and IPC target and 81-83 ºC for the male Y target.

2 Training Outline

2.1 Lesson Plan

Training can be modified based on level of participation in casework processing.

A. Technology Basis

1. Lysis Buffer: The Promega SwabSolution along with DTT is used to digest and release DNA from a variety of forensic samples such as blood, saliva, and semen after a short incubation.

2. Plexor HY System: The Plexor HY quantification kit is designed to quantify the concentration of amplifiable human (and higher primate) and male DNA in a sample. The results from the quantification can aid in determining if sufficient male DNA is present to proceed with traditional autosomal STR analysis.

B. Liquid Handling Robotics

1. Robot components

2. Deck set-up

3. Robotic software/scripts

C. Extraction Procedure

1. Role of lysis buffer and DTT

2. Use of reagent blanks
3. Documentation
4. Manual versus robotic set up of lysis

D. Quantification Procedure
1. Plexor HY protocol
2. Standard curves
3. Use of nontemplate controls
4. Manual versus robotic set up of quantification plate
5. Operation of 7500

E. Data Interpretation
1. Use of quantification values to determine if further processing is required
2. Melt curves
3. Autosomal/male DNA ratio
4. Reagent blanks versus non-template controls
5. Interpretation of the IPC

F. Identifying next step in SA kit processing
1. Negative results
2. Inconclusive results
3. Positive results
4. Autosomal vs. Y-STR testing
5. Additional evidence

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Plexor HY Technical Manual for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, current version

B. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Male Screening Process – Plexor HY

C. Texas DPS Austin/System Laboratory Male Screening Internal Validation,
1. May 2015, Valid-Method-AUS-DNA-MaleScreening-2015-0522
2. April 2017, Valid-Method-SYS-DNA-MaleScreening-CutOff-2017-0404

D. Local Male Screening Implementation Validation

E. SWGDAM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits in a Laboratory (most recent version)

F. Local policies/validations as applicable

G. Safety Data Sheets:
- Lysis buffer
- Plexor HY kit
- 5X Amp Solution
- DTT

3 Practice

3.1 Safety

Gloves must be worn during reagent preparation and testing. Clothing may protect unbroken skin; broken skin should be covered. Eye protection is recommended during reagent preparation and handling of liquids. Use universal precautions during evidence handling.

3.2 Standards, Controls, and Reagent Preparation

A. Controls
   1. Documentation and use of required forms
   2. Quantification blank and lysis blank as noted in the DNA SOP.

B. Reagent Preparation
   1. 1M Dithiothreitol
   2. Lysis master mix
   3. Quantification standard dilution series
   4. Quantification reaction mix formulation

3.3 Equipment

- AB Prism 7500
- 96 well optical reaction plates
- retainer base for plates
- optical adhesive covers/ applicator
- pipettors and pipette tips
- vortex
- microcentrifuge tubes
- microcentrifuge
- plate centrifuge or salad spinner
- Plexor HY Kit
- Lysis Buffer

3.4 Observed Performance

A. The trainee should observe the male screening sample preparation process of at least one sexual assault kit if they are not already trained on the process.

B. The trainee should observe the male screening process of a robotic plate, if they have not already been trained to perform this analysis.

C. The trainee should observe the male screening process of a manual plate set up if they seek qualification in manual methods.

D. Trainer or mentor will give instruction on the male screening method.

E. Trainer or mentor will give instruction on data analysis and interpretation including the use of validated quantification thresholds to determine if further sample analysis is needed.

F. Trainer or mentor will provide instruction and/or demonstrate the next steps in the casework workflow.

3.5 Supervised Performance

Trainee will prepare the lysis master mix, quantification standards and appropriate reaction mix formulation. This exercise is not required for trainees qualifying for interpretation only.

3.6 Independent Exercise

A. If qualification is sought for the male screening process using robotics:

1. Trainees that have been previously authorized to operate robotics involved in this process will perform a practice set of ten samples comprised of mixtures, single source, male and female samples that will be taken through the male screening process. Appropriate results must be obtained.

2. Trainees that have not been previously authorized to operate robotics involved in this process will have to successfully complete an abbreviated robotics training to include the following: Introduction to robotics, general robotic maintenance and deck set-up on robots involved in male screening. Once complete, a practice set of ten samples comprised of mixtures, single source, male and female samples will be taken through the male screening process. Appropriate results must be obtained.

B. If qualification is sought for the male screening process using manual methods trainee will perform a practice set of ten (10) samples comprised of mixtures, single source, male and female samples that will be taken through the male screening process using manual set up. Appropriate results must be obtained.

C. If qualification is sought for interpretation only there is no independent exercise for trainee to complete; the independent exercises for interpretation are completed during report writing in the *Male Screening Report Writing and Technical Review* module.

4 Assessment

4.1 Competency and Qualifying Examination

A. If qualification is sought for male screening process using robotics, a competency set of ten samples comprised of mixtures, single source, male and female samples will be successfully completed using the male screening method.

> ***Note:*** *If the trainee is qualifying for male screening preparation and male screening process simultaneously, the same samples may be used to satisfy both competency requirements.*

B. If qualification is sought for the male screening process using manual methods, a competency set of ten samples comprised of mixture, single source, male and female samples will be successfully completed using the male screening method using manual set up.

> ***Note:*** *The same samples may be used to satisfy competency requirements for robotic and manual processes.*

C. If qualification is sought for interpretation only, there is no practical competency test for the trainee to complete; the practical competency test for interpretation is completed during report writing in the *Male Screening Report Writing and Technical Review* module.

D. The trainer will administer a written examination; this examination is required for those trainees seeking qualification in the male screening process using robotics, male screening process using manual methods, and interpretation only. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent male screening processing of casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Male Screening Training Checklist (LAB-DNA-TM-03).

DNA-TM-04-03 MALE SCREENING REPORT WRITING AND TECHNICAL REVIEW

Purpose Provide trainee with guidelines for male screening casework report writing and for technical and administrative case reviews.

Prerequisite Introductory Forensic Biology/DNA Unit, Male Screening – Sample Preparation module, Male Screening Process module, General Laboratory Training Manual Fundamentals Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How the laboratory report is used to communicate to its reader both the analytical results and the conclusions of the analyst, conveying the essence of what would be said if the analyst were asked to give an opinion in court.

B. How the report is the method through which the analyst is able to state what the evidence means and also what it does not mean. It is highly desirable that the report be able to "stand alone" since decisions may be made by police officers, attorneys and the courts, based on the report, with no contact with the analyst.

C. How a thorough review of each case record is performed to ensure that conclusions and supporting data are reasonable and within the constraints of scientific knowledge.

D. The two separate review components to the case review process.

1. Technical Review

   a) How to conduct an evaluation of documentation to check for consistency, accuracy, and completeness.

   b) How to conduct an evaluation of reports, notes, data and other documents to ensure an appropriate and sufficient basis for the scientific conclusions.

   c) The requirements for personnel who perform technical review (must be a second qualified analyst.)

2. Administrative Review

   a) How to conduct a review of the report and supporting documentation to check for consistency with laboratory policies and for editorial correctness.

   b) The requirements for personnel who perform administrative review (must be conducted by someone other than the analyst completing the case.)

1.2 Practical

Following the completion of training the trainee will be able to:

1. Reduce technical data to a concise conclusion.
2. Write clear and concise reports that are provided to the submitting officer and prosecuting and defense attorneys.
3. Understand the purpose of case review.
4. Understand the requirements of the technical review and administrative review.

5. Technically review male screening cases from the testing performed to the written report.

2 Training Outline

2.1 Lesson Plan

A. Report writing

1. General case information
   - *a) Case documentation*
   - *b) Documentation, technical notes, case notes*
   - *c) Policy*
   - *d) Case-related correspondence*
   - *e) Records*
   - *f) Laboratory Reports*
   - *g) Elements in reports*
   - *h) Format of reports*
2. Submission information – Contains information about the evidence containers, date and method of submission, and relevant tracking information. Additional submissions must be clearly marked.
3. Requested analysis – Contains a general statement pertaining to the requested analysis, which can be a mixture of the laboratory's normally used statement and any special requests made by the submitting agency.
   - *a) Include a reference to relevant previous reports or reports previously issued in the same discipline.*
   - *b) Also, information regarding evidence transfer between laboratories or resubmissions may be included here.*
4. Evidence Description, Results of Analysis and Interpretation
   - *a) Listing of items analyzed for male screening analysis*
   - *b) Conclusions regarding samples that are negative for male DNA*
   - *c) Conclusions regarding samples that are positive for male DNA*
   - *d) Conclusions regarding samples that are inconclusive for male DNA*
   - *e) Conclusions regarding samples that are candidates for Y-STR analysis*
   - *f) Other examinations*
   - *g) Items that were not examined*
5. Investigative leads – This section is directed to the investigating officer to inform them of what additional evidence needs to be submitted in order for DNA analysis or other comparisons to be performed.
6. Disposition – Specific disposition notes should be used to inform the officer if any further action needs to be taken on their part regarding the evidence submitted. It is one way to identify items being retained in the laboratory. This section may also be used to indicate if evidence was forwarded to another lab, or section.

7. Other types of reports
   a) *Supplemental reports*
   b) *Amended reports*
   c) *Closed without Analysis reports*

B. Technical Review

1. The technical review will be performed on the final draft. The supporting case information should be organized in a logical fashion with case items highlighted on relevant forms.
2. The results, interpretations, opinions, and conclusions must be reasonable, within the constraints of validated scientific knowledge, and supported by the examination records.
3. The reporting statements should be written to ensure that results are communicated properly and clearly.
4. Refer to the Review of Laboratory Records chapter in the Crime Laboratory Service manual for the requirements of the technical review.
5. Technical review of batch records of analytical data may be conducted independently of the final review of the interpretations and report. If this approach is taken by the laboratory, the technical reviewer of the interpretation and report accepts the previous review of the batch paperwork and does not perform review of this part of the case record.

C. Administrative review

1. The administrative review will be performed on the final draft to ensure that the reported results are logical and accurate and there are no spelling or grammatical errors.
2. The chain of custody and disposition of the evidence will be reviewed for all evidence associated to the report.
3. Refer to the Review of Records chapter in the Crime Laboratory Service manual for the requirements of the administrative review.

D. Examination Counting

2.2 Required Readings

Readings are selected based on if the trainee seeks qualification in report writing, technical review, or both.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. <u>Report Writing</u>

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Review of Laboratory Records;*
   b) *Document Management and Deviations;*
   c) *Quality Incident (QI) and Quality Action Plan Process (QAP);*
   d) *Preventive Actions;*

e) *Laboratory Records;*
f) *Laboratory Reports, Letters, and Certificates;*
g) *Expunction and Destruction of Laboratory Records and Information;*
h) *Electronic Storage and Archival of Records;*
i) *Records Requests and Release of Laboratory Records and Information;*
j) *Evidence and Database Sample Integrity;*

2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Approved Standard Abbreviations List;*
   b) *Response to Quality Issues;*
   c) *Guidelines for Technical Case Review;*
   d) *Case Documentation;*
   e) *Report Writing Guidelines;*
   f) *Guidelines for Counting Examinations.*
3. Federal Bureau of Investigation. Quality Assurance Standards for Forensic DNA Testing Laboratories (most recent version). Standard 11.

B. Technical Review

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Review of Laboratory Records;*
   b) *Document Management and Deviations;*
   c) *Laboratory Records;*
   d) *Laboratory Reports, Letters, and Certificates;*
   e) *Evidence and Database Sample Integrity.*
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *Analytical Controls;*
   b) *Guidelines for DNA Technical Review;*
   c) *Case Documentation;*
   d) *Report Writing Guidelines.*
3. Federal Bureau of Investigation. Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (most recent version). Standard 12.
4. LIMS Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   a) *General;*
   b) *Male Screening Workflow.*

3 Practice

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

3.1 Safety

None

3.2 Standards, Controls, and Reagent Preparation

None

3.3 Equipment

None

3.4 Observed Performance

A. For report writing: the trainer will provide 10-20 cases involving male screening analysis (quantification results and interpretation) to the trainee to allow familiarity with the method of report writing in the laboratory.

B. For technical review: the trainer will demonstrate the technical review of at least one male screening report.

C. For batch review of technical records: the trainer will demonstrate the batch review of at least one male screening batch consisting of technical records from multiple cases.

3.5 Independent Exercises

A. To be considered qualified in report writing, the trainee must complete the following exercises, which also serve as the competency test in report writing:

1. The trainee will be provided with data (case report removed) from 5 male screening cases previously analyzed and reported.

   ***Note**: All case information including case number, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.*

2. Using the DNA SOP and instruction from the trainer, the trainee must provide an interpretation of the quantification results to include whether or not male DNA is present, if the data is inconclusive, and recommendations for downstream processing of the samples. Trainee will also prepare a report for each case according to the format in his/her laboratory.

3. Upon completion, the trainee may compare their statements with the actual statements reported on the case and discuss their conclusions/reports with the trainer.

B. To be considered qualified in technical review, the trainee must complete the following exercises, which also serve as the competency test in technical review:

1. The trainer will provide a minimum of 5 male screening cases on which the trainee will perform a preliminary technical review, prior to the actual case review by a

qualified analyst. These reviews shall be evaluated by the trainer, and the trainer will discuss any discrepancies found during the actual case review with the trainee.

***Note**: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.*

2. After an evaluation of the trainee's preliminary case reviews, the trainer and/or Technical Leader may assign further preliminary case reviews until several cases have been reviewed by the trainee without significant discrepancies between the actual case review.

C. To be considered qualified in batch review of technical records, the trainee must complete the following exercises, which also serve as competency test in batch review of technical records:

1. The trainer will provide at least 1 male screening batch of technical records on which the trainee will perform a preliminary batch review prior to the actual batch review by a qualified analyst. This review shall be evaluated by the trainer and the trainer will discuss any discrepancies found during the actual batch review with the trainee.

***Note**: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of batches reviewed and trainee's performance is included.*

2. After an evaluation of the trainee's preliminary batch review of technical records, the trainer and/or Technical Leader may assign further preliminary batch reviews until several batches have been reviewed by the trainee without significant discrepancies between the actual batch review.

4 Assessment

4.1 Competency and Qualifying Examination

A. The examination will consist of the independent exercises described above. This will serve as the competency test in report writing, case review, and batch review of technical records for male screening analysis.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent report writing and technical review for male screening analysis.

4.2 Evaluation of Training

The trainee and trainer will complete the Male Screening Training Checklist (LAB-DNA-TM-03).

05 ANALYTICAL DNA UNIT

DNA-TM-05-01 MANUAL DNA EXTRACTION

Purpose Enable trainee to recover and isolate DNA from a variety of forensic specimens

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How successful PCR amplification relies upon the isolation and purification of genomic DNA from forensic samples.

B. How extraction methods and purification techniques recover DNA and eliminate or minimize PCR amplification inhibitors arising from environmental insults and/or from substrates.

C. How 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 must be practiced during extraction procedures.

D. How 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.

E. The various extraction methods and purification techniques used on forensic specimens.

F. The purpose and benefits of the various DNA extraction methods performed in the laboratory.

G. Techniques used to increase the yield of DNA and to remove inhibitors of PCR amplification.

H. The quality control and precautionary measures associated with the DNA extraction methods utilized in the laboratory.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Prepare and store the necessary reagents used in DNA extractions.

B. Perform the necessary precautions to prevent contamination during extraction procedures.

C. Perform microscopic identification of spermatozoa as necessary.

D. Isolate DNA using various extraction procedures.

E. Perform the differential lysis extraction of semen stains.

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

2 Training Outline

2.1 Lesson Plan

A. Isolating DNA by organic extractions
   1. Purpose and benefits
   2. Role of Proteinase K, DTT, and detergents.
   3. Phenol/chloroform/isoamyl alcohol precautions
   4. Membrane filtration device(s)
   5. Digest Buffer (DB), Stain Extraction Buffer (SEB), TNE
   6. Methods
      a) Bloodstain extraction
      b) Buccal cell extraction
      c) Differential extraction (traditional and/or Erase)
      d) Hair root extraction
      e) Tissue extraction
      f) Bone/Teeth extraction
   7. Limitations

B. Isolating DNA by Chelex extractions
   1. Purpose and benefits
   2. Role of Proteinase K and DTT.
   3. Methods
      a) Bloodstain extraction
      b) Buccal cell extraction
      c) Hair root extraction
   4. Limitations

C. Isolating DNA by QIAamp DNA Mini Kit
   1. Purpose and benefits
   2. Role of Proteinase K, DTT, and detergents.
   3. Methods
      a) Bloodstain extraction
      b) Buccal cell extraction
      c) Differential extraction (traditional and/or Erase)
      d) Hair root extraction
      e) Bone/Teeth extraction
   4. Limitations

D. Isolating DNA by Prepfiler (manual extraction)
   1. Purpose
   2. Role of Proteinase K, DTT, and detergents.
   3. Methods
      a) *Bloodstain extraction*
      b) *Buccal cell extraction*
      c) *Differential extraction (traditional and/or Erase)*
      d) *Hair root extraction*
   4. Precautions
      a) *Guanadine Thiocyanate*
      b) *Guanadine salts*
   5. Limitations

E. Preparation of slides for microscopic identification of spermatozoa – Staining techniques (**optional** for technicians and analysts)

F. Microscopic Identification of Spermatozoa (**optional** for analysts)
   1. Microscopy review
   2. Sperm morphology
   3. Morphology of other common components of slides
   4. Interpretation of results

G. Inhibitors
   1. Mechanisms
      a) *Direct binding to DNA strand*
      b) *Direct interaction with polymerase*
   2. Types
      a) *Heme*
      b) *Dyes*
      c) *Soil*
      d) *Excess salt*
      e) *Ionic Detergents*
      f) *Alcohols (ethanol, phenol, isopropanol)*
   3. Methods to overcome inhibition
      a) *Swab transfer of material versus direct cutting*
      b) *Extraction methods*
      c) *Additional purification with QIAamp or Chelex*
      d) *Additional purification with membrane filtration devices*

e) Addition of BSA to PCR reactions

f) Choice of DNA polymerase used

g) Dilution

H. Quality control

1. Forms, worksheet documentation
2. Critical reagents
3. Reagent blanks
4. Separate extraction of known and questioned samples

I. Preserving extracted DNA

2.2 Required Readings

Readings are selected based on the extraction methods for which the trainee seeks qualification. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Butler John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.2.

B. Besetti, J. An Introduction to PCR Inhibitors. Promega, Inc., 2007.

C. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

1. Analytical Controls;
2. Response to Quality Issues;
3. Instruments and Equipment;
4. Physical Evidence Examination;
5. DNA Extraction and Purification section;
6. Reagent Preparation section;
7. Forms/Worksheets (as appropriate).

D. Walsh S, et al. PCR Inhibition and Bloodstains. Proceedings of the International Symposium on the Forensic. 1989.

E. Insert for membrane filtration devices (most recent version).

F. Safety Data Sheets for appropriate chemicals.

G. Any local policies/validations as applicable.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the method described in the reading.

A. Chelex – Walsh S, et al. Chelex 100 as Medium for Simple Extraction of DNA for PCR-Based Typing for Forensic Material. BioTechniques. 1991.

B. QIAamp – Insert for QIAamp DNA Mini Kit (most recent version).

C. PrepFiler

1. PrepFiler Forensic DNA Extraction Kit User Guide. Life Technologies (most recent version).
2. Brevnov MG, et al. Developmental Validation of the PrepFiler Forensic DNA Extraction Kit for Extraction of Genomic DNA from Biological Samples. J For Sci. 2009. 54(3), pp. 599-607.

D. Organic Extraction – Comey C, et al. DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis. J For Sci. 1994. 39. pp. 1254-1269.

E. Erase

1. Garvin, AM, Bottinelli M, Gola M, Conti A, and Soldati, G. DNA Preparation from Sexual Assault Cases by Selective Degradation of Contaminating DNA from the Victim. J For Sci. 2009. 54(6), pp. 1297-1303.
2. Insert for Erase Sperm Isolation kit.

F. Preparation of slides for microscopic identification of spermatozoa

1. Safety Data Sheets: aluminum sulfate, indigocarmine, nuclear fast red, picric acid or saturated picric acid solution
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

G. Microscopic Identification of Spermatozoa

1. Payne RL. Cytology for the forensic scientist. Unpublished. 1988.
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

3 Practice

3.1 Safety

A. Wear a lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

B. Use extreme caution when working with phenol/chloroform/isoamyl alcohol. Phenol/chloroform/isoamyl alcohol is toxic and causes burns and should only be used under a chemical fume hood and/or properly equipped laminar flow hood.

C. Picric acid, used in preparation of picroindigocarmine stain, is explosive when dry. Follow storage and preparation instructions and warnings carefully.

3.2 Standards, Controls, and Reagent Preparation

A. Controls

1. Documentation and use of required forms.
2. Reagent blanks for each set of extractions as noted in the DNA SOP.
3. Extraction kits as critical reagents

B. Reagent Preparation

1. Extraction reagents: stain extraction buffer, digest buffer, Chelex, TNE, Sarcosyl, Proteinase K, 0.39M and 1M Dithiothreitol, TE buffer as available.

2. Christmas tree stain (as applicable); nuclear fast red solution, picroindigocarmine solution
3. Refer to the DNA SOP, Reagents section

3.3 Equipment

- Scissors, scalpel, tweezers
- Cutting paper and/or glassine paper
- Pipettors and tips
- Microcentrifuge tubes, 1.5 mL, 2.0 mL, or spin basket tube
- Microcentrifuge
- Microscope
- Tube racks
- Vortex
- Water bath/dry bath incubator (56 +/- 1°C; 37°C +/- 1°C; and 70 +/- 1°C)
- Sterile toothpicks
- Sterile tissue pulverizer
- QIAamp kit
- Erase Sperm Isolation Kit
- Phenol:chloroform:isoamyl alcohol (v/v 25:24:1)
- Decapper
- Membrane filtration devices
- Boiling water bath
- Boileezers

3.4 Observed Performance

A. Trainer will give instruction and/or demonstrate various extraction and concentration techniques based on the methods for which the trainee seeks qualification.

B. Trainer will demonstrate the steps for separating epithelial cell DNA from sperm cell DNA using one of the extraction methods for which the trainee seeks qualification.

C. As applicable, trainer will demonstrate preparation of a microscope slide for identification of spermatozoa.

D. As applicable, trainer and trainee will discuss morphology of various cellular and acellular components in typical microscope slide preparations for sperm identification. Trainer will demonstrate the use of a microscope to identify spermatozoa.

E. Trainer will discuss the remaining extraction procedures in the DNA SOP.

F. Trainer will discuss proper documentation and QC of extracted samples.

3.5 Supervised Performance

A. Trainee will prepare various extraction solutions including Christmas Tree stain, if applicable.

B. Any reagents not available in the laboratory will be prepared as necessary by the trainee.

3.6 Independent Exercises

A. Trainee will manually extract the following practice samples using a variety of extraction techniques, dependent on manual extraction methods for which they seek qualification:

1. Five (5) bloodstains
2. Five (5) saliva swabs
3. Five (5) hair specimens
4. Five (5) mixed stains (sperm/epithelial cell)
   a) *Trainee will prepare microscope slides as part of differential extractions.*

B. As applicable, trainee will examine the stained slides from the differential extractions using normal light microscopy at the full range of magnification available.

1. Trainee will diagram, color and identify each part of a normal stained spermatozoon.
2. Trainee will diagram, color and identify each part of a normal stained epithelial cell.

C. These extractions will be frozen and may be used for quantitation and normalization exercises.

4 Assessment

4.1 Competency and Qualifying Examination

A. Trainee will manually extract the following DNA competency samples: 5 bloodstains, 5 buccal swabs, 5 mixed semen stains for differential extraction, and 5 hairs.

1. At least 5 samples must be performed per extraction method in which the trainee seeks qualification.

   ***Note**: the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the extraction procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst.*

2. If trainee has not previously qualified on preparation of microscope slides for identification of spermatozoa, the preparation of microscope slides during the differential extraction portion of the competency serves as a competency test in slide preparation.
3. If trainee has not previously qualified on microscopic identification of spermatozoa, the reading of slides and microscopic identification of spermatozoa during the differential extraction portion of the competency serves as a competency test in spermatozoa identification.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent manual extraction of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-02 MANUAL QUANTIFICATION

Purpose Enable trainee to quantify the amount of DNA recovered in extracted samples

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The necessity for DNA quantification of extracted samples prior to amplification.

B. Five methods for quantifying DNA and the advantages and limitations of each.

C. The basis for DNA quantification utilizing the Quantifiler Human and Quantifiler Duo kits.

D. The method of detection used with the Quantifiler Human and Quantifiler Duo kits and the advantages and limitations of each.

E. The quality control measures associated with the Quantifiler Human and Quantifiler Duo kits.

F. The purpose of the Internal PCR Control (IPC) and how to interpret the results.

G. How to recognize possible inhibition and how to proceed with inhibited samples.

H. How validated quantification thresholds are used to determine if further analysis is needed for samples.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Prepare dilutions of the human DNA standards as per directions in the Quantifiler Human DNA Quantification Kit and/or the Quantifiler Duo DNA Quantification Kit.

B. Use and maintain the AB 7500.

C. Prepare the master mix and perform the quantification set-up of samples for the Quantifiler Human and/or Quantifiler Duo kits.

D. Perform the quantification procedure on extracted DNA samples using the Quantifiler Human and/or Quantifiler Duo kits.

E. Calculate the male to female DNA ratio using the quantification values obtained from the Quantifiler Duo kit (**optional** depending on lab).

F. Apply quantification thresholds to data and determine when further analysis is needed based on these values.

G. Understand the requirements, procedure, and documentation necessary to perform the critical reagent quality control on any quantitation kit(s) the trainee will become competent in. (**Optional**: Perform the critical reagent quality control for these kits, as necessary in the trainee's lab).

1.3 Considerations

A. <u>Background</u>

1. Once DNA has been extracted from a sample, it is important to determine how much DNA is present before continuing with the amplification and typing process.

The polymerase chain reaction (PCR) short tandem repeat (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 ensure that sufficient DNA is added to the PCR reaction and will aid in troubleshooting if no PCR product is obtained. Quantification can also indicate the presence of PCR inhibitors and determine whether additional sample purification is necessary prior to the amplification step. It can also assist the analyst in determining which amplification chemistry is most appropriate for processing male to female mixtures. In addition, using minimal volumes of DNA extracts will maximize the number of genetic marker tests or repeat analyses that can be performed. Validated quantification thresholds can be used to determine if further processing of the sample is needed.

2. The 7500 Real Time PCR System can be used to determine the relative quantity of a target nucleic acid sequence in a sample by analyzing the cycle-to-cycle change in fluorescent signal as a result of amplification during a PCR.

3. The DNA quantification assay combines two 5' nuclease assays which are a target-specific human DNA only (Quantifiler) or human and human male DNA (Quantifiler Duo) assay and an internal PCR control (IPC) assay. The purpose of the IPC (Internal PCR Control) is to distinguish between true negative sample results and reactions affected by inhibitors, assay setup and chemistry/instrument failures.

B. Standard Curve

The standard curve is a graph of the C_T (cycle threshold) of quantification standard reactions plotted against the starting quantity of the standards. The software calculates the regression line by calculating the best fit with the quantification standard data points. Positive amplification occurs when the C_T value for the detector is <40. Because samples contain unknown amounts of DNA, a large range of C_T values is possible.

C. R^2 value

The R^2 value measures the closeness of fit between the standard curve regression line and the individual C_T data points of quantification standard reactions. An R^2 value ≥0.99 indicates a close fit.

D. Slope

The slope of this regression line indicates the PCR amplification efficiency for the assay. Slope values typically fall between -2.9 and -3.3. A slope of -3.3 indicates 100% amplification efficiency.

E. Y-intercept

The Y-intercept indicates the expected C_T value for a sample with quantity =1. Some variation in the Y-intercept value may be observed. These variations may be a result of: pipetting variation during standard setup, lot-to-lot variations in the kits, target-to-target variation, or instrument-to-instrument variation. Minor variations do not affect assay performance or quantification accuracy; however each lab should monitor the Y-intercept over time for each instrument to determine if variations become significant.

F. IPC

If the IPC amplification for certain samples appears reduced relative to IPC amplification for quantification standards, the sample is exhibiting partial PCR inhibition. If partial inhibition is

observed the analyst may proceed directly to an STR analysis or perform additional cleanup of the sample to remove the inhibitors. Additionally, the extract may be diluted and re-quantified prior to analysis.

1. **Negative Results:** No human DNA is detected when the IPC target amplified but the human-specific target (and/or male specific target) did not amplify.
2. **Invalid IPC Results:** If the human-specific target (and/or male-specific target) *and* the IPC target failed to amplify, this is highly indicative of complete PCR inhibition but it is not possible to distinguish between the absence of DNA, PCR reaction failure, and PCR inhibition.
3. **IPC Results Inconclusive:** With extremely high concentrations of human genomic DNA (>10 ng/µL), competition between the human-specific (and/or male-specific) and IPC PCR reactions appears to suppress IPC amplification for that sample. If the target amplifies with low C_T and high ΔR_n results, it is unlikely that PCR inhibitors are present. In these cases, appearance of suppression or failure of IPC amplification renders the IPC result inconclusive.
4. **PCR Inhibition:** Weak amplification (high C_T and low ΔR_n value) of the human target (and/or male-specific target) and no or weak amplification of the IPC may indicate PCR inhibition in the sample.

G. Quantifiler Human

1. The Quantifiler Human DNA Quantification Kit is designed to quantify the concentration of amplifiable human (and higher primate) DNA in a sample.
2. The following components of the IPC system are present in the Quantifiler PCR mix: Synthetic DNA template, Primers that hybridize specifically to the synthetic DNA template, and Probe labeled with VIC dye.
3. The IPC system template DNA is added to the reaction at a fixed concentration; therefore, the VIC C_T should range between **20** and **30.**

H. Quantifiler Duo (**optional** depending on lab)

1. The following components of the IPC system are present in the Quantifiler Duo Primer mix: Synthetic DNA template, Primers that hybridize specifically to the synthetic DNA template, and Probe labeled with NED dye.
2. Valid results are obtained when amplification is observed in both the Duo Human/Duo Male and the Duo IPC detectors.
3. The IPC system template DNA is added to the reaction at a fixed concentration; therefore, the NED C_T should range between **28** and **31**, with a variation of 1 C_T value across the standard curve samples.

2 Training Outline

2.1 Lesson Plan

A. Benefits of DNA Quantification

1. Choose appropriate DNA testing procedure
2. Preservation of sample for replicate analysis
3. Distinguish between inhibition and insufficient DNA quantity

4. PCR systems optimized for limited range of DNA quantity

B. Methods of DNA Quantification

1. UV Spectroscopy
   - *a) requires at least 2 µg DNA to detect*
   - *b) not primate specific*
2. Fluorometry
   - *a) requires at least 5 ng of DNA to detect*
   - *b) not primate specific*
   - *c) requires expensive instrumentation*
3. Yield Gel
   - *a) requires at least 2 ng of DNA to detect*
   - *b) cannot detect single stranded DNA*
   - *c) not primate specific*
4. Quantiblot
   - *a) sensitivity of 0.02 – 0.15 ng depending on detection method used*
   - *b) primate specific*
   - *c) can detect single stranded DNA*
5. Quantifiler Human and Quantifiler Duo
   - *a) .023 ng sensitivity*
   - *b) primate specific*
   - *c) can detect single stranded DNA*
   - *d) Quantifiler Duo can be used to determine the presence of male DNA*
6. Quantifiler Human Protocol
   - *a) Plate set-up and amplification*
   - *b) Detection*
   - *c) DNA quantity calculations by the software*
   - *d) Use of quantification values to determine if further processing is required*
   - *e) QC forms and documentation*
7. Quantifiler Duo Protocol (**optional** depending on lab)
   - *a) Plate set-up and amplification*
   - *b) Detection*
   - *c) DNA quantity calculations by the software*
   - *d) Male:Female DNA Ratio calculations*
   - *e) Use of quantification values to determine if further processing is required*
   - *f) QC forms and documentation*

8. AB 7500
   a) *Operating instructions*
   b) *Troubleshooting*
   c) *Cleaning, decontamination and maintenance*

2.2 Required Readings

Readings are selected based on the quantification kits for which the trainee seeks qualification. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. HID Real-Time PCR Analysis Software (v. 1.0, 1.2, or 1.3 depending on lab) for use with: 7500 Real-Time PCR Instruments. Life Technologies (most recent version).

B. Baechtel FS. The extraction, purification and quantification of DNA. Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. Quantico, Virginia, 1989, pp. 25-28.

C. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.3.

D. Manufacturer operating manuals relevant to the real-time instrument (most recent versions).

E. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory.
   1. Analytical Controls;
   2. Response to Quality Issues;
   3. Critical Reagents;
   4. Instruments and Equipment;
   5. Case Documentation;
   6. DNA Quantification section;
   7. Forms/Worksheets (as appropriate).

F. Li, R. Forensic Biology: Identification and DNA Analysis of Biological Evidence. 2008. CRC Press. Chapter 13.

G. Vallone, P. Human DNA Quantification Using Real-Time PCR Assays. NIST, 2009. (PowerPoint presentation).

H. Troubleshooting Quantifiler Kit Data from an FAS Perspective. Applied Biosystems. 03/2015.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the quantification kit described in the reading.

A. <u>Quantifiler Human</u>:
   1. Quantifiler Human and Y Human Male DNA Quantitation Kits User Guide. Life Technologies (most recent version).

2. Green RL, Ines CR, Boland C, and Hennessy LK. Developmental Validation of the Quantifiler Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples. J For Sci. 2005. 50, pp 809-824.
3. Local laboratory's Quantifiler validation(s).
4. Safety Data Sheet: Quantifiler Human
5. Local policies, as applicable.

B. Quantifiler Duo:

1. Quantifiler Duo DNA Quantification Kit User Guide. Life Technologies (most recent version).
2. Local laboratory's Quantifiler Duo validation(s)
3. Safety Data Sheet: Quantifiler Duo
4. Any local policies, as applicable.

3 Practice

3.1 Safety

Wear a lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

3.2 Standards, Controls, and Reagent Preparation

A. Controls

1. Documentation and use of required forms.
2. Quantification kits as critical reagents.

B. Reagent Preparation

1. Quantification standard dilution series
2. Reaction mix formulation

3.3 Equipment

- AB Prism 7500
- 96 well optical reaction plates
- Retainer base for plates
- Optical adhesive covers/ applicator
- Pipettors and pipette tips
- Vortex
- Microtubes – 0.5 ml
- Microcentrifuge
- Plate centrifuge or salad spinner
- Quantifiler Human DNA Quantification kit
- Quantifiler Duo DNA Quantification Kit
- TE buffer
- Glycogen (optional)

3.4 Observed Performance

A. Trainee will observe trainer setting up and running a Quantifiler Human and/or Quantifiler Duo plate.

B. Trainer will discuss proper documentation on worksheets and interpretation of results including the use of validated quantification thresholds to determine if further sample analysis is needed.

C. For Quantifiler Duo, trainer will discuss calculation of male to female DNA ratios.

D. Trainer will discuss background plates and appropriate computer maintenance.

3.5 Supervised Performance

A. Trainee will prepare the quantification standards and appropriate reaction mix formulation.

B. Trainee will run a background plate and perform appropriate computer maintenance. (**optional**)

3.6 Independent Exercises

A. If seeking qualification in Quantifiler Human quantification, the trainee will manually set up and run a Quantifiler Human plate on a minimum of 5 previously extracted practice samples (samples from modules *Manual DNA Extraction*, *Automated DNA Extraction - Prepfiler*, and *Automated DNA Extraction – Qiagen EZ1* may be used). Trainee will complete proper documentation of worksheets. If the trainee is a forensic scientist, they will also complete interpretation of results.

B. If seeking qualification in Quantifiler Duo quantification, the trainee will manually set up, run, and analyze a Quantifiler Duo plate on a minimum of 5 previously extracted samples (samples from modules *Manual DNA Extraction*, *Automated DNA Extraction - Prepfiler*, and *Automated DNA Extraction – Qiagen EZ1* may be used). Trainee will complete proper documentation of worksheets. If the trainee is a forensic scientist, they will also complete interpretation of results and calculation of male to female DNA ratios for these samples.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will manually set up and run a quantification plate on at least 10 competency samples using each of the kits for which qualification is sought.

> ***Note:*** *the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the quantification procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst.*

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent manual quantification of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-03 MANUAL AMPLIFICATION

Purpose Enable trainee to amplify DNA using validated amplification kits

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The steps involved in amplification.

B. The advantages and disadvantages of polymerase chain reaction (PCR)-based testing.

C. The different components in an amplification mix, as well as the purpose of each component.

D. The appropriate controls used in the amplification of DNA samples and their purposes.

E. The differences between the different validated amplification kits in the DPS Crime Laboratory system, as well as the advantages and limitations of each.

F. How to define and describe short tandem repeat (STR) markers.

G. How to define multiplexing and outline the STR markers contained in each amplification kit.

H. Possible causes of PCR inhibition and how to remove PCR inhibitors.

1.1 Practical

Upon completion of this training, the trainee will be able to:

A. Understand the procedure and documentation used to verify the performance of thermal cyclers. (**Optional**: Perform equipment quality control on the thermal cyclers).

B. Determine the appropriate amplification kit to utilize.

C. Determine the amount of template DNA necessary for amplification and prepare the appropriate dilutions of samples.

D. Prepare the master mix and perform the amplification set-up of samples.

E. Complete the amplification worksheet and appropriate documentation for quality control.

F. Understand the requirements, procedure, and documentation necessary to perform the critical reagent quality control on any amplification kit(s) the trainee will become competent in. (**Optional**: Perform the critical reagent quality control for these kits, as necessary in the trainee's lab).

1.2 Considerations

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. Different multiplex PCR systems also allow analysts to obtain DNA results from compromised samples that previously would have yielded limited or no genetic data and analysis of complex male:female DNA mixtures that often yield ambiguous or uninterpretable results with autosomal STRs.

2 Training Outline

2.1 Lesson Plan

A. General information on amplification

1. Advantages of PCR
   - *a) Amount of DNA template required*
   - *b) DNA degradation*
   - *c) Analysis time*
   - *d) Multiplexing*
2. Disadvantages and considerations of PCR
   - *a) Mixed samples*
   - *b) Preferential amplification and stochastic effects*
   - *c) Inhibition*
   - *d) Contamination*
3. Master mix ingredients
   - *a) Purpose of each component*
     - *i. DNA polymerase*
     - *ii. MgCl2*
     - *iii. deoxynucleotide triphosphates*
     - *iv. primers*
     - *v. Bovine serum albumin (BSA)*
   - *b) Modification of stringency of amplification by changing concentrations of certain components in master mix*
4. Steps involved in amplification
   - *a) Purpose of each step*
   - *b) Modification of stringency of amplification by changing times/temperatures of each step*
5. Appropriate controls
6. Thermal cycler quality control procedure

B. Background Information on STRs

C. General information on the amplification kits validated for use in the DPS Crime Laboratory system.

2.2 Required Readings

Readings are selected based on the amplification kits for which the trainee seeks qualification. It is required that all trainees undertaking this module qualify on the current validated autosomal STR amplification kit at a minimum. The readings in this section are required for all trainees.

Readings completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Investigator 24plex QS Handbook. Qiagen (most recent version)

B. Solution to overcome unusual shoulder formation at D2S441. Technical Note, Qiagen, May 2016

C. Product information updates for the Investigator 24plex QS and ESSplex SE QS Kits, Technical Note, Qiagen January 2017

D. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch. 4, 5, 10, and 13.

E. Butler J. Genetics and genomics of core short tandem repeat loci used in human identity testing. J For Sci. 2006. 51, pp. 253-265.

F. Manufacturer operating manuals relevant to the thermal cycler (most recent versions).

G. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

1. Facilities;
2. Analytical Controls;
3. Response to Quality Issues;
4. Critical Reagents;
5. Instruments and Equipment;
6. Case Documentation;
7. PCR Amplification System section;
8. Forms/Worksheets (as appropriate).

H. Kraemer, M. et al. Developmental validation of QIAGEN Investigator 24plex QS Kit and Investigator 24plex GO! Kit: Two 6-dye multiplex assays for the extended CODIS core loci. Forensic Science International: Genetics 2017. 29, pp 9-20.

I. Texas DPS and/or laboratory specific validation studies for approved amplification kits.

J. Safety Data Sheet: QIAGEN Investigator 24plex QS Kit.

K. Local policies, as applicable.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the amplification kit described in the reading.

A. Mini-STR:

1. AmpFlSTR MiniFiler PCR Amplification Kit User Guide. Life Technologies (most recent version).
2. Mulero J, et al. Development and Validation of the AmpF/STR MiniFiler PCR Amplification Kit: A MiniSTR Multiplex for the Analysis of Degraded and/or PCR Inhibited DNA. J For Sci. 2008. 53, pp. 838-852.
3. Safety Data Sheet: MiniFiler

B. Yfiler:

1. AmpFlSTR Yfiler PCR Amplification Kit User Guide. Life Technologies (most recent version).

2. Mulero J, et al. Development and Validation of the AmpF/STRs Yfiler PCR Amplification Kit: A Male Specific, Single Amplification 17 Y-STR Multiplex System. J For Sci. 2006. 51, pp. 64-75.

3. Mayntz-Press K, and Ballantyne J. Performance Characteristics of Commercial Y-STR Multiplex Systems. J For Sci. 2007. 52, pp. 1025-1034.

4. Safety Data Sheet: Yfiler.

C. Y23:

1. PowerPlex Y23 System Technical Manual. Promega (most recent version).

2. Thompson JM et al. Developmental validation of the PowerPlex Y23 System: A single multiplex Y-STR analysis system for casework and database samples. Forensic Science International: Genetics 2013. 7, pp 240-255.

3. Safety Data Sheet: Y23.

3 Practice

3.1 Safety

Wear lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

3.2 Standards, Controls, and Reagent Preparation

Controls

A. Documentation and use of required forms.

B. Amplification kits as critical reagents.

3.3 Equipment

- Calculator
- Microcentrifuge
- Microcentrifuge tubes, 1.5 mL and 2.0 mL
- 96 well plates
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Tube rack
- Investigator 24plex QS Amplification Kit
- AmpF/STR MiniFiler PCR Amplification Kit
- AmpF/STR Yfiler PCR Amplification Kit
- PowerPlex Y23 PCR Amplification Kit
- TE^{-4} buffer
- Glycogen (optional)

3.4 Observed Performance

A. To qualify in autosomal STR amplification

1. Trainee will observe trainer setting up and running an amplification using a validated autosomal STR amplification kit.
2. Trainer will discuss proper documentation on worksheets.

B. To qualify in mini-STR amplification:

1. Trainee will observe trainer setting up and running an amplification using a validated mini-STR kit.
2. Trainer will discuss proper documentation on worksheets.

C. To qualify in Y-STR amplification:

1. Trainee will observe trainer setting up and running an amplification using a validated Y-STR kit.
2. Trainer will discuss proper documentation on worksheets.

3.5 Independent Exercises

A. Trainee will manually normalize, set up, and run amplification on a minimum of 5 previously extracted and quantified practice samples using the currently validated autosomal STR amplification kit. Trainee will complete proper documentation of worksheets.

B. If seeking qualification for mini-STR amplification, trainee will manually normalize, set up and run amplification on a minimum of 5 previously extracted and quantified practice samples using the currently validated mini-STR amplification kit. Trainee will complete proper documentation of worksheets.

C. If seeking qualification for Y-STR amplification, trainee will manually normalize, set up and run amplification on on a minimum of 5 previously extracted and quantified practice samples using the currently validated Y-STR amplification kit for which qualification is sought. Trainee will complete proper documentation of worksheets.

D. For any of the independent exercises listed above, samples from modules *Manual DNA Extraction*, *Manual Quantification*, *Automated DNA Extraction - Prepfiler*, and *Automated DNA Extraction – Qiagen EZ1* may be used.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will manually normalize, set up, and run amplification on a minimum of 10 competency samples using the currently validated autosomal STR amplification kit.

B. The trainee will manually normalize, set up, and run amplification on a minimum of 10 competency samples using each additional amplification kit for which qualification is sought.

> ***Note****: the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the amplification procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst.*

C. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent manual amplification of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-04 LIQUID HANDLING ROBOTICS

Purpose Enable trainee to operate a robotic platform to perform DNA Quantitation setups, Normalization of samples, and PCR amplification setups. Detailed training of all analysts/technicians is not required for procedures that are not part of routine use (i.e. programming, calibration, cleaning).

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How liquid handling robots can be used in the laboratory to automate procedures by facilitating the transfer of multiple liquids for a given procedure.

B. How automation allows for more efficient use of human resources, increases processing speed, improves accuracy and precision, and alleviates analyst/technician fatigue.

C. The advantages and limitations of using liquid handling robotics.

D. Basic operation of the robotic platform and how it interrelates with EXCEL based data files.

E. The setup of the robotic platform.

F. The cleaning and maintenance of the robotic platform.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Setup the labware on the robotic deck for the appropriate test.

B. Perform cleaning of the robotic platform.

C. Calibrate the deck as appropriate.

D. Calibrate labware on the deck as appropriate.

E. Degas the system water as appropriate.

F. Operate the robot to perform DNA Quantitation, Normalization, PCR amplification setups, and CE plate setup as applicable.

G. Create the appropriate files from sample quantitation for use in the normalization and PCR setups as applicable.

2 Training Outline

2.1 Lesson Plan

A. Advantages of liquid handling robots

1. Accuracy and precision
2. Efficiency
3. Alleviate analyst/technician fatigue

B. Disadvantages of liquid handling robots
   1. Disassociation of the samples from the analyst/technician (this could also be seen as an advantage)
   2. Setup and programming can be difficult and time consuming
   3. Limitations of the robotic system

C. Calibrating the deck and labware, creating new labware (if applicable)
   1. When to calibrate
   2. Deck calibration
   3. Labware calibration
   4. Creating and defining new labware

D. Robot programming basics (if applicable)
   1. Adding labware to the deck
   2. Writing a basic program
      a) Flush/Wash
      b) Types of pipetting steps
      c) Aspirate/dispense
      d) Mapping

E. Operating the robot
   1. Degassing system water if applicable
   2. Verifying system fluid level if applicable
   3. Flushing the system and checking for bubbles if applicable
   4. Setting up the labware on the deck
      a) Relating physical deck location to the program
      b) Labware carriers
   5. Basic understanding of the test procedures
   6. Quantification
      a) Input of data from Quantification for Normalization
   7. Normalization and PCR setup
   8. CE plate setup
   9. Basic troubleshooting
   10. Quality Control

F. Cleaning and maintenance of the robot
   1. Changing the system fluid carboys, if applicable
   2. Flushing the system fluid, if applicable
   3. Cleaning the deck

4. Wash procedure, if applicable
5. Calibration
6. Preventative maintenance and performance checks

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   1. DNA Quantification – Quantifiler Using Tecan;
   2. DNA Amplification – Investigator 24plex QS Tecan;
   3. DNA Amplification – YFiler Tecan.
   4. CE Sample Setup – Sample Setup for Capillary Electrophoresis Using Qiagility Robot

B. Local documents pertaining to operation of the robotic platform.

C. Manufacturer operating manuals relevant to the robotic platform (most recent versions).

D. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.17.

E. Local laboratory's liquid handling robotics validation(s).

3 Practice

3.1 Safety

Wear lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

3.2 Standards, Controls, and Reagent Preparation

A. Controls

Documentation and use of required forms

B. Reagent Preparation
   1. If applicable, 35% methanol solution
   2. If applicable, 70% methanol solution

3.3 Equipment

- Microcentrifuge
- Microcentrifuge tubes, 1.5 mL, 0.5 mL
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Racks and carriers
- TE buffer

- 96 well plates
- Reagent troughs
- Strip caps and capping tool for 96 well plates (if applicable)
- Plate septa (if applicable)
- Methanol, Ethanol, or Isopropanol (as applicable)
- Roboscrub (as applicable)
- DI water
- Appropriate quantification and amplification kits
- Liquid handling robot

3.4 Observed Performance

A. Trainer will discuss proper operation of the liquid handling robot

B. If applicable, trainee will prepare 35% and 70% methanol solution.

3.5 Supervised Performance

Trainer will observe the trainee's performance in operating the robot.

3.6 Independent Exercises

A. If seeking qualification in quantitation, trainee will set up quantitation of at least 5 previously extracted practice samples using each quantitation kit and each liquid handling robotic platform for which they seek qualification.

B. If seeking qualification in amplification, trainee will set up normalization/amplification of at least 5 previously extracted practice samples using each amplification kit and each liquid handling robotic platform for which they seek qualification.

C. If seeking qualification in CE plate setup, trainee will perform CE plate setup on at least 5 previously amplified samples using each script on which qualification is sought (automated sample set up and/or individual sample setup).

4 Assessment

4.1 Competency and Qualifying Examination

A. Trainee will set up a sample plate of a minimum of 10 competency samples for each robotic platform using each process for which qualification is sought. For CE plate setup, competency samples consist of at least 5 samples setup using each script on which qualification is sought for a total of 10 samples.

> ***Note****: the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the automated procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst*.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent use of liquid handling robotics for set up of quantitation and normalization/amplification of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-05 AUTOMATED DNA EXTRACTION – PREPFILER

Purpose Enable trainee to recover and isolate DNA from a variety of forensic specimens using PrepFiler chemistry on the Tecan Freedom Evo automated platform.

Prerequisite Introductory Forensic Biology/DNA Unit and the Liquid Handling Robotics module (The Liquid Handling Robotics module may be undertaken simultaneously with this module.)

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How isolation and purification of genomic DNA can affect the success of PCR amplification.

B. How the PrepFiler extraction method recovers DNA and eliminates or minimizes PCR amplification inhibitors arising from environmental insults and/or from substrates.

C. How automated extraction methods are designed to maximize throughput while minimizing the possibility of contamination.

D. Stringent precautionary measures and quality control that must be practiced during extraction procedures due to the sensitivity of PCR and the risk of evidentiary samples, particularly those with small amounts of DNA, becoming contaminated.

E. The use of filter concentration, Chelex clean-up, and Qiagen clean-up procedures to concentrate and further purify PrepFiler extracts.

F. The purpose, benefits, and limitations of the automated PrepFiler DNA extraction method compared to the manual extraction methods performed in the laboratory.

G. The quality control and precautionary measures associated with the automated PrepFiler DNA extraction method.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Prepare and store the necessary reagents used in automated PrepFiler DNA extractions.

B. Perform the necessary precautions to prevent contamination.

C. Perform microscopic identification of spermatozoa as necessary.

D. Setup and perform daily quality control procedures on the Tecan Freedom Evo.

E. Isolate DNA using the automated PrepFiler DNA extraction procedures.

F. Perform and document the necessary quality control measures practiced during automated PrepFiler DNA extractions.

2 Training Outline

2.1 Lesson Plan

A. Isolating DNA using automated PrepFiler extraction method, purpose, benefits, disadvantages

1. Reagent Preparation

a) Wash buffers

b) *Elution buffer*

c) *Magnetic beads*

d) *1M DTT*

e) *Lysis buffer*

f) *Isopropanol (also referred to as binding solution)*

g) *TE^{-4}*

2. Sample Lysis Setup

a) *Manual*

b) *Automated (**optional**)*

3. Robot Operation

a) *Daily flush*

b) *Tightening LiHa cones and syringes*

c) *Cleaning LiHa cones*

d) *Setting up labware/reagents*

e) *Opening/operating scripts*

4. Troubleshooting

a) *Robot errors*

b) *Visually confirming liquid transfers (tube to deep well plate, deep well plate to 96-well plate)*

c) *Qualification in manual Prepfiler in case automated process fails*

B. Preserving extracted DNA

C. Preparation of slides for microscopic identification of spermatozoa – Staining techniques (**optional** for technicians and analysts)

D. Microscopic Identification of Spermatozoa (**optional** for analysts)

1. Microscopy review
2. Sperm morphology
3. Morphology of other common components of slides
4. Interpretation of results

2.2 Required Readings

Readings are selected based on the extraction methods for which the trainee seeks qualification. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. PrepFiler User Bulletin. Validation of the PrepFiler Automated Forensic DNA Extraction Kit on the Tecan HID EVOlution-Extraction System. Applied Biosystems, Foster City, CA, December 2008.

B. PrepFiler Automated Forensic DNA Extraction Kit. Life Technologies (most recent version).

C. Brevnov MG, et al. Developmental Validation of the PrepFiler Forensic DNA Extraction Kit for Extraction of Genomic DNA from Biological Samples. J For Sci. 2009. 54(3), pp. 599-607.

D. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). PrepFiler DNA Extraction.

E. Shewale JG and Lui RH. Forensic DNA Analysis: Current Practices and Emerging Technologies. 2014. CRC Press. Section 3.3 Automated Extraction of DNA pp 49-55.

F. Local laboratory's implementation validation(s) for the PrepFiler extraction method and liquid handling robotics.

G. Local policies, as applicable.

H. Garvin, AM, Bottinelli M, Gola M, Conti A, and Soldati, G. DNA Preparation from Sexual Assault Cases by Selective Degradation of Contaminating DNA from the Victim. J For Sci. 2009. 54(6), pp. 1297-1303. (**optional depending on lab**).

I. Insert for Erase Sperm Isolation kit (**optional depending on lab**).

J. Safety Data Sheets for PrepFiler and associated extraction chemicals.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the method described in the reading.

A. Preparation of slides for microscopic identification of spermatozoa

1. Safety Data Sheets: aluminum sulfate, indigocarmine, nuclear fast red, picric acid or saturated picric acid solution.
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

B. Microscopic Identification of Spermatozoa

1. Payne RL. Cytology for the forensic scientist. Unpublished. 1988.
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

3 Practice

3.1 Safety

A. Wear lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

B. Use extreme caution when working with the lysis buffer as it contains guanidine thiocyanate. Guanidine thiocynate is highly incompatible with bleach, acids, and other bases. Guanidine thiocynate must be disposed of properly.

C. Magnetic particles are an irritant and incompatible with bleach.

D. Wash buffer is an irritant.

E. Ethanol and isopropanol are irritants and flammable.

F. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

G. Picric acid, used in preparation of picroindigocarmine stain, is explosive when dry. Follow storage and preparation instructions and warnings carefully.

3.2 Standards, Controls, and Reagent Preparation

A. Controls

1. Documentation and use of required forms.
2. Reagent blanks for each set of extractions as noted in the DNA SOP.
3. Extraction kits as critical reagents

B. Reagent Preparation

1. Activation of magnetic particles
2. 1M Dithiothreitol, Wash Buffer A, Wash Buffer B, TE^{-4}
3. Christmas tree stain (as applicable); nuclear fast red solution, picroindigocarmine solution
4. Refer to the DNA SOP

3.3 Equipment

- Scissors, scalpel, plastic tweezers, or toothpicks
- Pipettors and pipette tips
- Microcentrifuge tubes or prepfiler columns
- Microscope
- Spin baskets
- Microcentrifuge
- Vortex
- 1M Dithiothreitol
- Shaking incubator
- Isopropanol (ACS reagent grade, >= 99.5%)
- Ethanol (Molecular biology grade 95%)
- Liquid handling (LiHa) disposable tips (DiTis) with filters for Tecan (1000 and 200 μL)
- 100 mL disposable troughs for reagents
- 96 well reaction plate
- 96 well magnetic ring stand
- 96 well deep-well plate
- PrepFiler Automated Forensic DNA Extraction Kit
- Erase Sperm Isolation Kit
- Tecan Freedom Evo robotic platform

3.4 Observed Performance

A. Trainer will give instruction and/or demonstrate various extraction and concentration techniques.

B. Trainer will discuss proper documentation and QC of extracted samples.

C. As applicable, trainer will demonstrate preparation of a microscope slide for identification of spermatozoa.

D. As applicable, trainer and trainee will discuss morphology of various cellular and acellular components in typical microscope slide preparations for sperm identification. Trainer will demonstrate the use of a microscope to identify spermatozoa.

3.5 Supervised Performance

A. Trainee will prepare all reagents including Christmas Tree stain, if applicable.

B. Any reagents not available in the laboratory will be prepared as necessary by the trainee.

3.6 Independent Exercises

A. Trainee will extract the following practice samples using the automated PrepFiler extraction method as appropriate:

1. Five (5) bloodstains
2. Five (5) saliva swabs
3. Five (5) hair specimens
4. Five (5) differential specimens (traditional and/or Erase)

   Trainee will prepare microscope slides as part of differential extractions.

B. As applicable, trainee will examine the stained slides from the differential extractions using normal light microscopy at the full range of magnification available.

1. Trainee will diagram, color and identify each part of a normal stained spermatozoon.
2. Trainee will diagram, color and identify each part of a normal stained epithelial cell.

C. These extractions will be frozen and may be used for quantitation and normalization exercises.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will extract (at a minimum) the following competency samples using the automated PrepFiler extraction method as appropriate to the sample types extracted in their laboratory: five bloodstains, five saliva swabs, five hair specimens, five differential specimens.

***Note**: the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the extraction procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst.*

1. If trainee has not been previously qualified on preparation of microscope slides for identification of spermatozoa, the preparation of microscope slides during the

differential extraction portion of the competency serves as a competency test in slide preparation.

2. If trainee has not been previously qualified on microscopic identification of spermatozoa, the reading of slides and microscopic identification of spermatozoa during the differential extraction portion of the competency serves as a competency test in spermatozoa identification.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent automated PrepFiler extraction of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-06 AUTOMATED DNA EXTRACTION – QIAGEN EZ1

Purpose Enable trainee to recover and isolate DNA from a variety of forensic specimens using the Qiagen EZ Investigator chemistry on the Qiagen EZ1 XL Advanced automated platform.

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How successful PCR amplification relies upon the isolation and purification of genomic DNA from forensic samples.

B. How the Qiagen EZ1 Investigator extraction method recovers DNA and eliminates or minimizes PCR amplification inhibitors arising from environmental insults and/or from substrates.

C. The stringent precautionary measures and quality control that are practiced during extraction procedures due to the sensitivity of PCR and the risk of contamination of evidentiary samples, particularly those with small amounts of DNA.

D. How automated extraction methods are designed to maximize throughput while minimizing the possibility of contamination.

E. How the Qiagen EZ1 Investigator extraction purification methods utilize filter concentration and are comparable to current manual Qiagen extraction methods.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Prepare and store the necessary reagents used in automated Qiagen EZ1 Investigator DNA extractions.

B. Perform the necessary precautions to prevent contamination.

C. Perform microscopic identification of spermatozoa as necessary.

D. Setup and perform daily quality control procedures on the Qiagen EZ1.

E. Isolate DNA using the automated Qiagen EZ1 Investigator DNA extraction procedures.

F. Perform and document the necessary quality control measures practiced during automated Qiagen EZ1 Investigator DNA extractions.

2 Training Outline

2.1 Lesson Plan

A. Isolating DNA using the Qiagen EZ1 Investigator extraction method, purpose, benefits, and disadvantages

1. Reagent Preparation
   - *a) 1M DTT*
   - *b) Carrier RNA*

2. Sample Lysis Setup
   a) *Bloodstain, cigarette butt, envelope flap, and stain on fabric extraction*
   b) *Forensic surface and contact swab/stain extraction*
   c) *Differential extraction*
   d) *Hair root extraction*
   e) *Nail scrapings and chewing gum extraction*
3. Using carrier RNA
4. Instrument Operation
   a) *Setting up worktable*
   b) *Loading reagents/samples*
   c) *Operating protocols*
      i. *Trace protocol*
      ii. *Large Volume protocol*
      iii. *Tip Dance protocol*
   d) *Daily/Weekly Maintenance*

B. Preparation of slides for microscopic identification of spermatozoa – Staining techniques (**optional** for technicians and analysts)

C. Microscopic Identification of Spermatozoa (**optional** for analysts)
   1. Microscopy review
   2. Sperm morphology
   3. Morphology of other common components of slides
   4. Interpretation of results

D. Preserving extracted DNA

2.2 Required Readings

Readings are selected based on the extraction methods for which the trainee seeks qualification. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. EZ1 DNA Investigator Handbook (most recent version).

B. EZ1 Advanced XL User Manual (most recent version).

C. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Qiagen EZ1 Extraction.

D. Local laboratory's implementation validation(s) for the EZ1 extraction method and liquid handling robotics.

E. Local policies, as applicable.

F. Montpetit SA, Fitch IT, and O'Donnell PT. A Simple Automated Instrument for DNA Extraction in Forensic Casework. J For Sci. 2005. 50(3), pp. 555-563.

G. Kishore RK, Hardy WR, Anderson VJ, Sanchez NA, Buoncristiani MR. Optimization of DNA Extraction from Low-Yield and Degraded Samples Using the BioRobot EZ1 and BioRobot M48. J For Sci. 2006. 51(5), pp. 1055-1061.

H. Safety Data Sheets for DNA extraction chemicals associated with the EZ1

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the method described in the reading.

A. Preparation of slides for microscopic identification of spermatozoa

1. Safety Data Sheets: aluminum sulfate, indigocarmine, nuclear fast red, picric acid or saturated picric acid solution
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

B. Microscopic Identification of Spermatozoa

1. Payne RL. Cytology for the forensic scientist. Unpublished. 1988.
2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Spermatozoa Examination.

3 Practice

3.1 Safety

A. Wear lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

B. Use extreme caution when working with the reagent cartridges as they contain guanidine hydrochloride and guanidine thiocyanate. Guanidine hydrochloride and guanidine thiocyanate are incompatible with bleach, acids, and other bases. Guanidine hydrochloride and guanidine thiocyanate must be disposed of properly.

C. Picric acid, used in preparation of picroindigocarmine stain, is explosive when dry. Follow storage and preparation instructions and warnings carefully.

D. Proteinase K is an irritant.

E. Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Appropriate personal protective equipment must be work during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

3.2 Standards, Controls, and Reagent Preparation

A. Controls

1. Documentation and use of required forms
2. Reagent blanks for each set of extractions as noted in the DNA SOP.

B. Reagent Preparation

1. 1M Dithiothreitol, carrier RNA
2. Christmas tree stain (as applicable); nuclear fast red solution, picroindigocarmine solution

3. Refer to the DNA SOP

3.3 Equipment

- Scissors, scalpel, tweezers
- Pipettors and pipette tips
- Microcentrifuge tubes or spin basket tubes
- Spin baskets
- Microcentrifuge
- Microscope
- Vortex
- 1M Dithiothreitol
- Wet/dry incubator
- Shaking incubator
- Qiagen Buffer ATL
- Qiagen Buffer MTL
- Qiagen EZ1 Investigator Kit which contains reagent cartridges, disposable tip holders, disposable filter tips, sample tubes, elution tubes, Buffer G2, Proteinase K, and Carrier RNA
- Qiagen EZ1 Advanced XL robotic workstation

3.4 Observed Performance

A. Trainer will give instruction and/or demonstrate various extraction and concentration techniques.

B. Trainer will discuss proper documentation and QC of extracted samples.

C. As applicable, trainer will demonstrate preparation of a microscope slide for identification of spermatozoa.

D. As applicable, trainer and trainee will discuss morphology of various cellular and acellular components in typical microscope slide preparations for sperm identification. Trainer will demonstrate the use of a microscope to identify spermatozoa.

3.5 Supervised Performance

A. Trainee will prepare all reagents including Christmas Tree stain if applicable.

B. Any reagents not available in the laboratory will be prepared as necessary by the trainee.

3.6 Independent Exercises

A. Trainee will extract, at a minimum, the following practice samples using the Qiagen EZ1 Investigator extraction method as appropriate:

1. Five (5) bloodstains
2. Five (5) saliva swabs
3. Five (5) hair specimens
4. Five (5) differential specimens

Trainee will prepare microscope slides as part of differential extractions.

B. As applicable, trainee will examine the stained slides from the differential extractions using normal light microscopy at the full range of magnification available.

1. Trainee will diagram, color and identify each part of a normal stained spermatozoon.
2. Trainee will diagram, color and identify each part of a normal stained epithelial cell.

C. These extractions will be frozen and may be used for quantitation and normalization exercises.

D. The extraction exercise listed above may be modified based on prior experience of the trainee.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will extract, at a minimum, the following competency samples using the Qiagen EZ1 Investigator extraction method as appropriate to the sample types extracted in the trainee's laboratory: five bloodstains, five saliva swabs, five hair specimens, five differential extractions.

***Note**: the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the extraction procedure(s) was performed successfully. Therefore, the samples must be processed through the remainder of the DNA analysis steps either by the trainee or by another qualified analyst.*

1. If trainee has not previously qualified on preparation of microscope slides for identification of spermatozoa, the preparation of microscope slides during the differential extraction portion of the competency serves as a competency test in slide preparation.
2. If trainee has not previously qualified on microscopic identification of spermatozoa, the reading of slides and microscopic identification of spermatozoa during the differential extraction portion of the competency serves as a competency test in spermatozoa identification.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent automated extraction of DNA casework samples using the EZ1.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

DNA-TM-05-07 CAPILLARY ELECTROPHORESIS INSTRUMENT OPERATION AND TROUBLESHOOTING

Purpose Educate trainee on the proper use of the CE instrument and collection software, how to troubleshoot problems with the instrument and how to make recommendations on fixing any encountered problems

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The software utilized by the CE instrument.

B. The proper way to care for and clean the CE instrument.

C. The steps to take in troubleshooting the CE instrument.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Perform capillary electrophoresis on previously extracted, quantified, and amplified DNA product.

B. Use the CE instrument software to maintain the instrument, run the instrument, and collect data.

C. Clean the CE instrument and demonstrate proper care of the CE instrument.

D. Observe the evaluation, application, and documentation of a spectral.

E. Discuss data archival and maintenance on the instrument computer.

1.3 Considerations

A. Spatials

1. The genetic analyzer software uses images collected during a spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls and is detected by the CCD camera.

2. A spatial calibration is required when a capillary array has been installed or replaced, when the capillary array has been temporarily removed from the detection block, and when the instrument has been moved.

B. Spectrals

1. Amplification kits use specific dye chemistry to label fragments. A spectral must be run annually for each dye chemistry used in the laboratory.

   a) A six dye system is used for Qiagen chemistries which includes 6-FAM, BTG, BTY, BTR2, BTP, and BTO ILS.

   b) A five dye system is used for AB chemistries which includes 6-FAM, VIC, NED, PET, and LIZ ILS.

   c) A five dye system is used for Promega chemistries includes Fluorescein, JOE, TMR-ET, CXR-ET, and WEN ILS.

2. A spectral file is generated by running matrix standards for each dye chemistry. The matrix standard is a standardized set of DNA fragments labeled with each of the dyes.
3. The collection software produces a matrix table based on the data from the spectral run. The rows and columns of the matrix table represent the virtual filters and the dye-labeled PCR products respectively.
4. Spectral files vary between instruments, virtual filter sets, and run conditions on a single instrument. However, the spectral overlap between the dyes is very reproducible under constant run conditions.

C. Internal lane standards (ILS)

Internal size standards are essential in STR analysis and must be run with every sample.

1. The internal lane size standards are DNA fragments of known size used to normalize injection-to-injection migration differences and are needed to determine the size of each of the unknown DNA fragments.
2. The internal lane standards utilized by the laboratory are GeneScan (GS) LIZ size standard for Life Technologies chemistries, BTO ILS for Qiagen chemistries and WEN ILS for Promega chemistries.

D. Allelic Ladders

The allelic ladder is created by the kit manufacturer and is specific to each amplification kit.

1. Ladders provide a reference DNA size for the alleles included in the ladder mixture. After the base pair size has been assigned to a peak using the size standard, the software will label that peak with a repeat number (allele) corresponding to the same base pair size peak in the ladder.
2. At least one ladder must be present in each run, as they adjust for different sizing measurements obtained from different instruments and run conditions.

2 Training Outline

2.1 Lesson Plan

A. General Information on the instrument computer

1. Archiving data
2. Computer maintenance

B. General information on the CE instrument

1. CE instrument tutorial on computer
2. Parts of the instrument
   - *a) Laser*
   - *b) CCD*
   - *c) Mother board*
   - *d) Autosampler*
   - *e) Heat block*
   - *f) Pump block*

3. Instrument break-down and cleaning
    a) *Reasons*
    b) *Frequency*
    c) *Pump block*
    d) *Water/buffer vials and septa*
    e) *Capillary storage*
4. Rebooting the instrument
    a) *Soft reboot*
    b) *Hard reboot*
    c) *Resetting the instrument*

C. Sample preparation/electrophoresis
1. Spatials and spectrals
    a) *Purpose of spatials and spectrals*
    b) *Making a spatial and spectral*
    c) *Spectral failure*
2. Instrument set-up
    a) *Running Wizards*
    b) *Filling the pump block with polymer*
    c) *Making buffer*
    d) *Replacing buffer and water*
3. Plate record
    a) *Protocols and modules*
    b) *Dye filter set*
    c) *Injection time*
    d) *Polymer type*
    e) *Array length*
4. Sample set-up
    a) *Internal lane size standards and formamide*
    b) *Adding sample*
    c) *Denaturing and quick freeze (**optional** step depending on laboratory validation)*

D. Documentation of maintenance of the CE instrument and computer
1. Capillary changes
2. Polymer lot # and expiration date
3. Internal lane size standard lot #
4. Analysis parameters

5. Spectral files
6. Spatial files
7. Hard drive error check
8. Data back-up

E. Troubleshooting
1. Identifying issues with data
2. Determining causes of issues
   a) *Urea crystals*
   b) *Formation of formate ions*
   c) *Bubbles*
   d) *Breakdown of buffer vial septa*
   e) *Old buffer*
   f) *Bad injections*
   g) *Bad laser*
   h) *Bad CCD camera*
   i) *Dirty capillary window or bad capillary*
   j) *Arcing*
   k) *Temperature fluctuations*
3. Determining solutions to various issues that may arise

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Butler John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch. 6.

B. DNA Standard Operating Procedures, Texas Department of Public Safety Crime Laboratory (most recent version).
1. Facilities;
2. Analytical Controls;
3. Instruments and Equipment;
4. Case Documentation;
5. Capillary Electrophoresis;
6. Forms/Worksheets (as appropriate).

C. DNA System/Laboratory Validations, Texas Department of Public Safety Crime Laboratory.

D. Any local policies as applicable.

E. Getting Started Guide for Genetic Analyzers. Life Technologies (most recent/applicable version).

F. Maintenance, Troubleshooting, and Reference Guide for Genetic Analyzers. Life Technologies (most recent/applicable version), Chapters 1-5, 11.

G. DNA Fragment Analysis by Capillary Electrophoresis User Guide. Life Technologies (most recent/applicable version), Chapters 1-4 and 11.

H. Using Data Collection Software User Bulletin. Life Technologies (most recent/applicable version).

I. Investigator 24plex QS Handbook. Qiagen (most recent version).

J. AmpF/STR MiniFiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (**optional** depending on lab)

K. AmpF/STR Yfiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (**optional** depending on lab)

L. PowerPlex Y23 System Technical Manual. Promega (most recent version). (**optional** depending on lab)

M. Shewale JG and Liu RH. Forensic DNA Analysis: Current Practices and Emerging Technologies. 2014. CRC Press. Automated Extraction of DNA, sections 7.3-7.8, pp 137-156.

N. Safety Data Sheets:
- formamide;
- polymer;
- 10x Genetic analyzer buffer with EDTA

3 Practice

3.1 Safety

A. Read SDS's for chemicals used in laboratory. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

B. Formamide is harmful if absorbed through the skin and is considered an irritant. Polymers are considered irritants.

3.2 Standards, Controls, and Reagent Preparation

A. Standards and Controls

1. The appropriate amplification controls, the in-lane size standard, and a ladder must be included on each run.
2. The ladder and in-lane size standard are necessary for genotyping.
3. The appropriate amplification controls, the in-lane size standard, and the ladder ensure that the instrument is working properly and analyzing data appropriately.
4. Completion of required forms and proper documentation – refer to DNA SOP.

B. Reagent Preparation

1x Genetic Analyzer buffer

3.3 Equipment

- AB Prism CE Genetic Analyzer (Instrument, computer and appropriate software)
- AB Prism CE Genetic Analyzer capillary arrays
- AB Prism CE Genetic Analyzer septa/plates
- Genetic Analyzer buffer vials/reservoirs and septa
- Freezer or ice bath
- Heat block or thermal cycler
- Internal lane size standard appropriate for amplification kit use
- AB Prism 10X Genetic Analyzer Buffer w/ EDTA
- Matrix standard kit appropriate for amplification kit used
- Hi-DI formamide
- Performance Optimized Polymer (POP-4 polymer)
- Vortex
- Microcentrifuge tubes -1.5 mL
- Pipettes
- Pipette tips
- Decapper

3.4 Observed Performance

Trainer will demonstrate proper technique for cleaning and setting up the CE instrument and troubleshooting the instrument.

3.5 Independent Exercises

A. Trainee will set up and run previously amplified samples on the CE instrument.

B. Trainee will troubleshoot any issues with the CE instrument and suggest/implement ways to remedy issues.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will set up and run a minimum of 10 competency samples for each instrument series in which qualification is sought.

> ***Note:*** *the only way to grade the competency set is to use the final profiles developed from the competency to confirm that the CE set up was performed successfully. Therefore, the samples must be analyzed in the DNA analysis software either by the trainee or by another qualified analyst.*

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent operation of CE instruments for developing profiles from DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the Analytical DNA Unit Training Checklist (LAB-DNA-TM-04).

06 DNA INTERPRETATION UNIT

DNA-TM-06-01 THEORY OF ANALYTICAL DNA

Purpose Enable trainees that interpret DNA results but either have not completed the analytical DNA portion of the training manual or have no prior analytical forensic DNA experience to understand the underlying theory behind the analytical DNA process used to generate data for interpretation.

Prerequisite Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of the underlying theory behind the analytical DNA process used to generate the data they are interpreting. This module is intended for those trainees that did not complete the Analytical DNA Unit training and have not had prior experience with DNA extraction, quantification, and amplification.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. DNA Extraction

1. Describe and be familiar with the various extraction methods and purification techniques used on forensic specimens.
2. Understand the purpose, benefits, and limitations of the various DNA extraction methods performed in the laboratory.
3. Describe techniques to increase the yield of DNA and to remove inhibitors of PCR amplification.
4. Understand the purpose, benefits, and limitations of the automated PrepFiler and Qiagen EZ1 Investigator DNA extraction methods compared to the manual extraction methods performed in the laboratory.
5. Understand the quality control and precautionary measures associated with the DNA extraction methods utilized in the laboratory.

B. DNA Quantification

1. Understand the necessity for DNA quantification of extracted samples prior to amplification.
2. Describe 5 methods for quantifying DNA and the advantages and limitations of each.
3. Understand and explain the basis for DNA quantification utilizing the Quantifiler Human and Quantifiler Duo kits.
4. Describe the method of detection used with the Quantifiler Human and Quantifiler Duo kits and the advantages and limitations of each.
5. Understand the quality control measures associated with the Quantifiler Human and Quantifiler Duo kits. Understand the purpose of the Internal PCR Control (IPC) and how to interpret the results.
6. Recognize possible inhibition and how to proceed with inhibited samples.

C. DNA Amplification

1. Describe the steps involved in amplification.
2. Outline the advantages and disadvantages of polymerase chain reaction (PCR)-based testing.
3. Describe the different components in an amplification mix, as well as the purpose of each component.
4. Describe the appropriate controls used in the amplification of DNA samples and their purposes.
5. Describe the differences between the different validated amplification kits in the DPS Crime Laboratory system, as well as the advantages and limitations of each.
6. Assess if the appropriate amount of template DNA was used for amplification
7. Define and describe short tandem repeat (STR) markers.
8. Define multiplexing and outline the STR markers contained in each amplification kit.
9. Describe possible causes of PCR inhibition and how to remove PCR inhibitors.

D. Robotics

1. Describe how automated methods for extraction, quantification, and amplification processes are designed to maximize throughput while minimizing the possibility of contamination.
2. Describe how automation allows for more efficient use of human resources, increases processing speed, improves accuracy and prevision, and alleviates analyst/technician fatigue.
3. Understand the advantages and limitations of using robotics.

2 Training Outline

2.1 Lesson Plan DNA Extraction

A. Isolating DNA by organic extractions

1. Purpose, benefits
2. Role of Proteinase K, DTT, and detergents.
3. Membrane filtration device(s)
4. Digest Buffer (DB), Stain Extraction Buffer (SEB), TNE
5. Methods
   - *a) Bloodstain extraction*
   - *b) Buccal cell extraction*
   - *c) Differential extraction(traditional and/or Erase)*
   - *d) Hair root extraction*
   - *e) Tissue extraction*
   - *f) Bone/Teeth extraction*

6. Limitations

B. Isolating DNA by Chelex extractions

1. Purpose, benefits
2. Role of Proteinase K and DTT.
3. Methods
   - *a) Bloodstain extraction*
   - *b) Buccal cell extraction*
   - *c) Hair root extraction*
4. Limitations

C. Isolating DNA by QIAamp DNA Mini Kit

1. Purpose, benefits
2. Role of Proteinase K, DTT, and detergents.
3. Methods
   - *a) Bloodstain extraction*
   - *b) Buccal cell extraction*
   - *c) Differential extraction (traditional and/or Erase)*
   - *d) Hair root extraction*
   - *e) Bone/Teeth extraction*
4. Limitations

D. Isolating DNA by Prepfiler (automated and manual extraction)

1. Purpose
2. Methods
   - *a) Bloodstain extraction*
   - *b) Buccal cell extraction*
   - *c) Differential extraction (traditional and/or Erase)*
   - *d) Hair root extraction*
3. Role of Proteinase K, DTT, and detergents.
4. Limitations

E. Isolating DNA using the Qiagen EZ1 Investigator extraction method

1. Purpose
2. Methods
   - *g) Bloodstain extraction*
   - *h) Buccal cell extraction*
   - *i) Differential extraction*
   - *j) Hair root extraction*

2. Role of Proteinase K, DTT, carrier RNA, and detergents.
3. Limitations
4. Membrane filtration devices: concentration and purification of samples
5. Quality control
   a) *Forms, worksheet documentation*
   b) *Critical reagents*
   c) *Reagent blanks*
   d) *Separate extraction of known and questioned samples*

2.2 Lesson Plan – DNA Quantification

A. Benefits of DNA quantification
   1. Choose appropriate DNA testing procedure
   2. Preservation of sample for replicate analysis
   3. Distinguish between inhibition and insufficient DNA quantity
   4. PCR systems optimized for limited range of DNA quantity

B. Methods of DNA Quantification
   1. UV Spectroscopy
      a) *Requires at least 2 µg DNA to detect*
      b) *Not primate specific*
   2. Fluorometry
      a) *Requires at least 5 ng of DNA to detect*
      b) *Not primate specific*
      c) *Requires expensive instrumentation*
   3. Yield Gel
      a) *Requires at least 2 ng of DNA to detect*
      b) *Cannot detect single stranded DNA*
      c) *Not primate specific*
   4. Quantiblot
      a) *Sensitivity of 0.02 – 0.15 ng depending on detection method used*
      b) *Primate specific*
      c) *Can detect single stranded DNA*
   5. Quantifiler Human and Quantifiler Duo
      a) *0.023 ng sensitivity*
      b) *Primate specific*
      c) *Can detect single stranded DNA*
      d) *Quantifiler Duo can be used to determine the presence of male DNA*

C. Basis of DNA quantification using Quantifiler Human and Quantifiler Duo

1. Assay components

   a) *Synthetic DNA template*

   b) *Primers for target (human only or human and human male)*

   c) *Primers for IPC*

   d) *DNA standards*

   e) *Reaction mix*

2. Evaluation of standard curve

   a) *How curve is made*

   b) *Slope*

   c) *R2 value*

   d) *Intercept*

3. Application of standard curve to data

   a) *C_T cycle threshold*

   b) *Determination of DNA quantities*

4. Assessing the IPC

   a) *Negative result: IPC has result but human/male target did not amplify*

   b) *Invalid result: IPC and human/male target did not amplify*

   c) *Inconclusive result: IPC did not amplify but human/male target has robust ampificaton*

   d) *PCR inhibition: IPC has weak result or did not amplify and human/male target has weak amplification*

2.3 Lesson Plan – Amplification

A. General information on amplification

1. Advantages of PCR

   a) *Amount of DNA template required*

   b) *DNA degradation*

   c) *Analysis time*

   d) *Multiplexing*

2. Disadvantages and considerations of PCR

   a) *Mixed samples*

   b) *Preferential amplification and stochastic effects*

   c) *Inhibition*

   d) *Contamination*

3. Purpose of each component in the master mix

   a) *DNA polymerase*

b) $MgCl_2$

c) deoxynucleotide triphosphates

d) primers

e) Bovine serum albumin (BSA)

f) Modification of stringency of amplification by changing concentrations of certain components in master mix

4. Steps involved in amplification

a) Purpose of each step

b) Modification of stringency of amplification by changing times/temperatures of each step

5. Appropriate controls

6. Amount of template DNA necessary for amplification

B. Background Information on STRs

C. General information on the amplification kits validated for use in the DPS Crime Laboratory system.

2.4 Lesson Plan Robotics

A. Advantages of robotics

1. Accuracy and precision

2. Efficiency

3. Alleviate analyst/technician fatigue

B. Disadvantages of robotics

1. Disassociation of the samples from the analyst/technician (this could also be seen as an advantage)

2. Setup and programming can be difficult and time consuming

3. Limitations of the robotic system

2.5 Required Readings

Readings are selected based on the methods that are used in the trainee's laboratory. Readings marked as required are required for all trainees. Those readings marked as conditional are required if the method is used in the trainee's laboratory.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. General:

DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory. (required)

1. Analytical Controls;

2. Response to Quality Issues;

3. Critical Reagents,

4. Instruments and Equipment;
5. Case Documentation;
6. DNA Extraction and Purification section;
7. DNA Quantification section;
8. PCR Amplification System section;
9. Forms/Worksheets (as appropriate).

B. DNA Extraction:

1. Butler John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.2. (required)
2. Besetti, J. An Introduction to PCR Inhibitors. Promega, Inc., 2007. (required)
3. Walsh S, et al. Chelex 100 as Medium for Simple Extraction of DNA for PCR-Based Typing for Forensic Material. BioTechniques. 1991. (conditional - Chelex)
4. Insert for QIAamp DNA Mini Kit (most recent version). (conditional - QIAamp)
5. PrepFiler Forensic DNA Extraction Kit User Guide. Life Technologies (most recent version). (conditional - PrepFiler)
6. PrepFiler User Bulletin. Validation of the PrepFiler Automated Forensic DNA Extraction Kit on the Tecan HID EVOlution-Extraction System. Applied Biosystems, Foster City, CA, December 2008. (conditional – PrepFiler)
7. Shewale JG and Lui RH. Forensic DNA Analysis: Current Practices and Emerging Technologies. 2014. CRC Press. Section 3.3 Automated Extraction of DNA pp 49-55. (conditional – PrepFiler)
8. EZ1 DNA Investigator Handbook (most recent version). (conditional – EZ1)
9. Montpetit SA, Fitch IT, and O'Donnell PT. A Simple Automated Instrument for DNA Extraction in Forensic Casework. J For Sci. 2005. 50(3), pp. 555-563. (conditional – EZ1)
10. Comey C, et al. DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis. J For Sci. 1994. 39. pp. 1254-1269. (conditional – any organic extraction method)
11. Insert for Erase Sperm Isolation kit (conditional - Erase)
12. Any local policies/validations as applicable.

C. DNA Quantification:

1. Quantifiler Human and Y Human Male DNA Quantitation Kits User Guide. Life Technologies (most recent version). (conditional – Quantifiler Human)
2. Quantifiler Duo DNA Quantification Kit User Guide. Life Technologies (most recent version). (conditional – Quantifiler Duo)
3. Baechtel FS. The extraction, purification and quantification of DNA. Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. Quantico, Virginia, 1989, pp. 25-28. (required)

4. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.3. (required)
5. Li, R. Forensic Biology: Identification and DNA Analysis of Biological Evidence. 2008. CRC Press. Chapter 13. (required)
6. Vallone, P. Human DNA Quantification Using Real-Time PCR Assays. NIST, 2009. (PowerPoint presentation). (required)
7. Troubleshooting Quantifiler Kit Data from an FAS Perspective. Applied Biosystems. 03/2015. (required)
8. Any local polices/validations as applicable.

D. DNA Amplification:

1. Investigator 24plex QS Handbook. Qiagen (most recent version) (required)
2. Solution to overcome unusual shoulder formation at D2S441. Technical Note, Qiagen, May 2016 (required)
3. Product information updates for the Investigator 24plex QS and ESSplex SE QS Kits, Technical Note, Qiagen January 2017 (required)
4. AmpFlSTR MiniFiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (conditional – MiniFiler [mini-STR])
5. AmpFlSTR Yfiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (conditional – Yfiler [Y-STR])
6. PowerPlex Y23 System Technical Manual. Promega (most recent version). (conditional – Y23 [Y-STR])
7. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch. 4, 5, 10, and 13. (required)
8. Butler J. Genetics and genomics of core short tandem repeat loci used in human identity testing. J For Sci. 2006. 51, pp. 253-265. (required)
9. Mayntz-Press K, and Ballantyne J. Performance Characteristics of Commercial Y-STR Multiplex Systems. J For Sci. 2007. 52, pp. 1025-1034. (conditional – Yfiler [Y-STR])
10. Any local policies/validations as applicable.

E. Robotics:

1. Manufacturer operating manuals relevant to the robotic platform used (most recent versions).
2. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch. 17.
3. Any local policies/validations as applicable.

3 Practice

3.1 Safety

Wear lab coat and gloves when working in the laboratory. Safety glasses or goggles may be required during some operations.

3.2 Standards, Controls, and Reagent Preparation

A. For Extraction Observation:

1. Controls

   a) *Documentation and use of required forms.*

   b) *Reagent blanks for each set of extractions as noted in the DNA SOP.*

   c) *Extraction kits as critical reagents.*

2. Reagent Preparation

   a) *Extraction reagents: stain extraction buffer, digest buffer, Chelex, TNE, Sarcosyl, Proteinase K, 0.39M and 1M Dithiothreitol, TE buffer as available.*

   b) *Refer to the SOP, Reagents section*

B. For Quantification Observation:

1. Controls

   a) *Documentation and use of required forms.*

   b) *Quantification kits as critical reagents.*

2. Reagent Preparation

   a) *Quantification standard dilution series*

   b) *Reaction mix formulation for quantification.*

C. For Amplification Observation:

1. Controls

   a) *Documentation and use of required forms.*

   b) *Amplification kits as critical reagents.*

2. Reagent Preparation – None

D. For Robotics Observation:

1. Controls

   a) *Documentation and use of required forms.*

   b) *Reagent blanks for each set of extractions as noted in the DNA SOP.*

2. Reagent Preparation

   a) *If applicable, 35% methanol solution.*

   b) *If applicable, 70% methanol solution.*

   c) *Activation of magnetic particles as appropriate.*

   d) *1M Dithiothreitol, Wash Buffer A, Wash Buffer B, TE-4 as applicable.*

   e) *Carrier RNA as applicable.*

3.3 Equipment

A. For Extraction Observation:
- Scissors, scalpel, tweezers
- Cutting paper and/or glassine paper
- Pipettors and tips
- Microcentrifuge tubes, 1.5 mL, 2.0 mL, or spin basked tube
- Microcentrifuge
- Tube racks
- Vortex
- Water bath/dry bath incubator
- Sterile toothpicks
- Sterile tissue pulverizer
- QIAmp kit
- Erase Sperm Isolation Kit
- Phenol: chloroform:isoamyl alcohol (v/v 25:24:1)
- Decapper
- Membrane filtration devices
- Boiling water bath
- Boileezers

B. For Quantification Observation:
- AB Prism 7500
- 96 well optical reaction plates
- Retainer base for plates
- Optical adhesive covers/applicator
- Pipettors and pipette tips
- Vortex
- Microtubes – 0.5 mL
- Microcentrifuge
- Place centrifuge or salad spinner
- Quantifiler Human DNA Quantification kit
- Quantifiler Duo DNA Quantification kit
- TE^{-4} buffer
- Glycogen (optional)

C. For Amplification Observation:
- Calculator
- Microcentrifuge
- Microcentrifuge tubes, 1.5 mL and 2.0 mL
- 96 well plates

- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Thermal cycler
- Amplification tubes, 0.2 mL
- Tube rack
- Investigator 24plex QS Amplification Kit
- AmpF/STR MiniFiler PCR Amplification Kit
- AmpF/STR Yfiler PCR Amplification Kit
- PowerPlex Y23 PCR Amplification Kit
- TE^{-4} buffer
- Glycogen (optional)

E. For Robotics Observation:

- Microcentrifuge
- Microcentrifuge tubes, 1.5 mL, 0.5 mL
- Spin baskets
- Pipette tips
- Pipettors, adjustable
- Tube decapper
- Vortex
- Racks and carriers
- TE^{-4} buffer
- 96 well plates
- Reagent troughs
- Strip caps and capping tool for 96 well plates (if applicable)
- Methanol, Ethanol, or Isopropanol (as applicable)
- Roboscrub (as applicable)
- DI water
- Appropriate extraction, quantification and amplification kits
- Appropriate liquid handling robot/robotic workstation
- Shaking incubator (if applicable)
- 96 well magnetic ring stand (if applicable)
- 96 well deep-well plate (if applicable)
- Wet/dry incubator (as applicable)
- 1M Dithiothreitol (as applicable)
- Qiagen Buffer ATL (as applicable)
- Qiagen Buffer MTL (as applicable)

3.4 Observed Performance

A. Extraction

1. Trainer will discuss proper extraction techniques based on which methods are used in the trainee's laboratory.
2. Trainee should observe the extraction process for each of the extraction techniques used in the laboratory (**optional**).

B. Quantification

1. Trainer will discuss proper quantification techniques based on which methods are used in the trainee's laboratory.
2. Trainee should observe the quantification process for each of the quantification techniques used in the laboratory (**optional**).

C. Amplification

1. Trainer will discuss proper amplification techniques based on which methods are used in the trainee's laboratory.
2. Trainee should observe the amplification process for each of the amplification techniques used in the laboratory (**optional**).

D. Robotics

1. Trainer will discuss proper operation of robotics based on which platforms are used in the trainee's laboratory.
2. Trainee should observe the operation of the robot(s) for each automated process used in the laboratory (**optional**).

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

B. Successful completion of this module is determined by the trainer and is a prerequisite for independent interpretation of DNA results unless the trainee has completed the Analytical DNA portion of the training manual or has previous experience with analytical DNA processes in a forensic DNA laboratory.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-02 DATA ANALYSIS AND SAMPLE CHARACTERIZATION

Purpose Educate trainee on how to use data analysis software to produce electropherograms, how to interpret electropherogram data from capillary electrophoresis runs, and options for troubleshooting data

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived)

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How capillary electrophoresis works with the data collection software to generate raw data.

B. The components used by DNA analysis software for analysis of raw data.

C. How to use the DNA analysis software to analyze data from the CE instrument.

D. The criteria for determining if a profile is single source or mixture and partial or full.

E. Evaluation of the data to recognize artifacts in a profile.

F. Determining peak height ratios (PHR) for single source profiles.

G. How to recognize the need for troubleshooting for a sample.

H. The importance and difference between an analytical and stochastic threshold.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Explain how capillary electrophoresis instruments collect data.

B. Analyze and interpret samples using the DNA analysis software.

C. Use the DNA analysis software to analyze raw data and obtain electropherograms.

D. Troubleshoot problem data and give the possible causes and recommend solutions to remedy the problem.

E. Examine electropherograms to differentiate between artifacts (such as stutter, minus A, spikes and pull-up) and true alleles.

F. Properly apply analytical thresholds that are specific to amplification kits used in the laboratory.

G. Properly apply a stochastic threshold to a profile.

H. Characterize samples as partial or full, single source or mixture.

I. Discuss data archival and maintenance on the analysis computer.

1.3 Considerations

A. Background

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.

2. Data gathered by the instrument must be analyzed by qualified personnel. The comparisons drawn from the data are used to identify inclusions and/or exclusions. This enables the analyst to determine whether or not a DNA profile could or could not have originated from an individual.

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

B. Spectrals

1. Multicomponent analysis is the process that separates the different fluorescent dye colors into distinctive spectral components.

   a) *A six dye system is used for Qiagen chemistries which includes 6-FAM, BTG, BTY, BTR2, BTP, and BTO ILS.*

   b) *A five dye system is used for AB chemistries which includes 6-FAM, VIC, NED, PET, and LIZ ILS.*

   c) *A five dye system is used for Promega chemistries includes Fluorescein, JOE, TMR-ET, CXR-ET, and WEN ILS.*

2. Although each dye emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra.

3. The precise spectral overlap is measured by analyzing a standardized set of DNA fragments labeled with each of the dyes. These dye-labeled fragments are matrix standards. The rows and columns of the matrix table represent the virtual filters and the dye-labeled PCR products respectively.

4. Spectral files vary between instruments, virtual filter sets, and run conditions on a single instrument. However, the spectral overlap between the dyes is very reproducible under constant run conditions.

5. If excessive artifacts such as pull-up are observed in samples during a capillary electrophoresis run, a new spectral is recommended. A spectral must be run after planned maintenance, and a spectral must be run after a capillary change in 3500 series instruments.

C. Internal lane standards (ILS)

1. Internal size standards are essential in STR analysis and are run with every sample.

2. The internal lane size standards are DNA fragments of known size used to normalize injection-to-injection migration differences, thereby providing excellent sizing precision within a set of capillary injections.

3. The internal lane standards utilized by the laboratory are GeneScan (GS) LIZ size standard for Life Technologies chemistries, BTO ILS for Qiagen chemistries and WEN ILS for Promega chemistries.

   a) *Investigator 24plex QS (Qiagen chemistry): The DNA analysis software uses the size standard to assign base pair sizes to each peak in a sample using the Local Southern Sizing method. This method utilizes two BTO peaks above and two*

below each possible allele sized. The size standard is used in conjunction with Investigator 24plex QS, and an analysis range of 60 to 500 bp is required for proper sizing.

b) Minifiler (Life Technology chemistry): The DNA analysis software uses the size standard to assign base pair sizes to each peak in a sample using the Third Order Least Squares method. This method uses regression analysis to make a best-fit size-calling curve. It is used to determine sizes extending beyond the physical range of the size standard.The size standard is used in conjunction with Minifiler, and an analysis range of 65 to 400 bp is required for proper sizing.

c) Yfiler (Life Technology chemistry): The DNA analysis software uses the size standard to assign base pair sizes to each peak in a sample using the Local Southern Sizing method. The size standard is used in conjunction with Yfiler, and an analysis range of 75 to 400 bp is required for proper sizing.

d) Y23 (Promega chemistry): The DNA analysis software uses the size standard to assign base pair sizes to each peak in the sample using the Local Southern Sizing method. The size standard is used in conjunction with Y23 and a partial analysis range of 60-500 bp is required for proper sizing.

D. Allelic Ladders

The allelic ladder is created by the kit manufacturer and is specific to each amplification kit.

1. Ladders provide a reference DNA size for the alleles included in the ladder mixture. After the base pair size has been assigned to a peak using the size standard, the software will label that peak with a repeat number (allele) corresponding to the same base pair size peak in the ladder.
2. Fragments of similar base pair sizes from different loci are also distinguished by the wavelength of the fluorescent dye used to tag the primers in the amp kit, allowing for multicomponent analysis.
3. At least one ladder must be present in each run, as they adjust for different sizing measurements obtained from different instruments and run conditions.

E. Amplification Controls

1. A positive and negative amplification control must be run on each project.
2. Both controls can be useful for troubleshooting problems with amplification and electrophoresis.
3. The positive control must exhibit the profile in the Analytical Controls chapter of the DNA SOP.
4. The purpose of the positive control is to show that the amplification reagents work and also to show that the genotyping software is functioning properly.
5. The negative control must not have any peaks below analytical threshold and primer peaks must be observed in the raw data.
6. The purpose of the negative control is to show that the amplification reagents do not introduce exogenous DNA into samples.

F. Genotyping

1. When analyzing results, allele calls are assigned to sample peaks by comparing their sizes to those obtained for the known alleles in the allelic ladders. Thus, a kit-appropriate ladder must be present within each run.

2. Each ladder used for analysis must have the appropriate number of alleles present for each locus when analyzed. (See DPS system validated amplification kit manuals for appropriate alleles in the respective ladder.)

3. Bin sets are used to provide reference allele sizes for alleles that are physically present in the ladder and some less common alleles that are not present in the ladder. The bins allow for allele calls to be correctly assigned because the bin takes into account a ±0.5 bp size difference. This corrects for possible differences in migration between the ladders and the samples.

4. The DNA analysis software is automated genotyping software used for analysis of data collected on a CE Instrument.

5. All values for the DNA analysis software will be set to the default settings, with the exception of analysis ranges and threshold settings. Process Quality Values (PQVs) will not be used to replace individual data analysis.

6. Samples from the plate manager or each injection list will be run through the DNA analysis software without manipulation of the imported data or the subsequent results and/or allele designations determined by the DNA analysis software.

G. STR anomalies

1. Stutter

   a) *A stutter peak is typically a reproducible minor product peak one repeat shorter (N-4 for example for a tetranucleotide repeat) or in some cases one repeat unit more (N+4 for example) than the corresponding main allele peak that is produced during amplification of STR loci.*

   b) *Stutter products may be caused by slippage of the DNA polymerase during amplification, probably due to out-of-alignment re-annealing of complementary target sequences during extension or out-of-alignment re-annealing of incomplete PCR products prior to extension.*

   c) *Stutter products may occur at 8bp, or very rarely 2bp or 12bp, shorter than the true allele and may be observed at 4bp greater than the main allele peak in rare instances or with overloaded samples for tetranucleotide repeats.*

   d) *Trinucleotide repeats may produce stutter at n-3. Stutter percentages vary by locus.*

2. Minus A/Plus A

   a) *The DNA polymerase used in STR/PCR amplification catalyzes the addition of a single nucleotide to the 3' ends of double stranded PCR products. This non-template addition results in a PCR product that is one base pair longer than the actual target DNA sequence.*

   b) *STR/PCR amplifications have been optimized to favor the "A" nucleotide addition.*

   c) *Incomplete "A" nucleotide addition may occur when too much amplification product is generated due to over addition of template DNA.*

3. Spectral Pull-up

Pull-up is identified as a minor peak of the same base pair size as the true allele but in a different color. Pull-up occurs when too much template DNA has been added to the amplification mix or when a faulty spectral is being utilized.

4. Spikes

Spikes are narrow peaks that are typically present in at least two colors, have the same data points, and are generally not reproducible.

5. Microvariants and Off-ladder alleles

a) *Microvariant alleles contain only a portion of one of their repeat units. As a result, their sizing will fall somewhere between that of alleles containing full repeats.*

b) *The number designation for alleles that contain a microvariant that falls within the range spanned by the ladder alleles should include the number of complete repeats followed by a decimal point and the number of base pairs in the incomplete repeat. For example, an allele labeled as 9.3 will contain 9 copies of the repeat unit plus 3 more base pairs from that repeat unit.*

c) *Off-ladder alleles that are not labeled by the genotyping software must be verified by re-injection of the sample or by confirmation due to their presence in multiple samples from the same origin. Because these alleles are not labeled by the genotyping software, a calculation is required to determine the allele designation.*

d) *Calculate off-ladder alleles for autosomal samples using the following formulae and document the result in the case record. If off-ladder alleles are homozygous, the allele from the nearest locus will be used as the sister allele.*

δ_1 = the difference between the ladder allele x (L_x) and the sample sister allele (S_x) to the OL allele at any given allele x

δ_2 = The difference between the nearest ladder allele y (L_Y) and the sample OL allele (S_{OL})

C = The relative peak shift of the OL allele from the nearest ladder allele

$\delta_1 = S_x - L_x$

$\delta_2 = S_{OL} - L_Y$

$C = |\delta_1 - \delta_2|$ in basepairs

e) *Calculate off-ladder alleles for Y-STR samples using the following formulae and document the result in the case record. For ladder alleles (L_Y), it is acceptable to use an average basepair size from all ladders in the run or to use the basepair size from a single ladder.*

L_Y = the nearest ladder allele y

S_{OL} = sample OL allele

C = The relative peak shift of the OL allele from the nearest ladder allele

$C = |S_{OL} - L_Y|$

f) *For both autosomal and Y-STR samples, if an allele falls outside the smallest or largest allele in the ladder at a given locus, the allele will be designated as greater than or less than the appropriate ladder allele (i.e., >13.3 for TH01) and no calculations are required.*

g) *Microvariants and off-ladder alleles are used for statistical calculations.*

6. Tri-Allelic Loci

Tri-allelic patterns must be verified by either re-injection, re-amplification or by observation of their presence in multiple samples from the same origin. Tri-allelic loci are used for comparisons, but cannot be used for single-source statistical calculations.

2 Training Outline

2.1 Lesson Plan

A. Capillary Electrophoresis

1. Multicomponent analysis
2. Separation of DNA fragments
3. Detection by the instrument

B. General Information on the analysis computer

1. Archiving data and computer maintenance
2. Raw data versus project data

C. DNA analysis software

1. Software Set up
   - a) *Panels*
   - b) *Bins*
   - c) *Stutter settings*
2. Analysis parameters
   - a) *Setting threshold values*
   - b) *Light smoothing*
   - c) *Analysis range and excluding primer peak*
   - d) *Sizing method: local southern or third order least squares for sizing*
3. Examination of Raw Data
   - a) *Allelic Ladder*
   - b) *Internal lane size standard*
     - i. *Defining the internal lane size standard*
     - ii. *Examining the 246 base pair fragment (LIZ 500 only) for troubleshooting*
   - c) *Offsets*
   - d) *Virtual alleles*
   - e) *Primer peaks in raw data*

4. Analyzing Data
   a) *Labeling peaks with base pair sizing, peak height, etc.*
   b) *Extra peaks in the electropherogram*
      i. *Off-ladder peaks*
      ii. *Spikes*
      iii. *Pull-up*
      iv. *Stutter*
      v. *Minus A*
      vi. *Kit related artifacts*
   c) *Microvariants/Off-ladder Alleles*
      i. *Sizing*
      ii. *CODIS entry when outside the allelic ladder*
   d) *Tri-Allelic loci*
   e) *Allelic Ladders*
   f) *Positive Control*
   g) *Negative Control*
   h) *Reagent Blanks*
   i) *Sample assessment – Re-injections*

D. Analysis of electropherogram data
   1. Analytical and Stochastic Thresholds
      a) *The analytical threshold for the Texas DPS system was calculated by the average baseline noise peak height of amplification blanks and/or samples plus 10 standard deviations of those peak heights.*
      b) *The stochastic threshold was calculated by creating a dilution series of decreasing DNA template volumes to evaluate where allelic, but not locus drop-out occurred. Then the average surviving sister allele height of a heterozygous locus was calculated plus 3 standard deviations.*
   2. Stochastic effect and allelic dropout
      a) *Degraded DNA*
      b) *Low template DNA*
      c) *Partial profiles*
      d) *Inhibition/partial inhibition*
   3. Dilution Studies
      a) *DNA concentration requirements*
      b) *Sensitivity within each CE instrument*
      c) *Balance across loci*
      d) *Heterozygous peak height balance*

4. Characterization
   a) *Single source – Requirements for full vs partial vs uninterpretable*
   b) *Mixtures*
      i. *Peak height ratios (heterozygous peak height balance)*
      ii. *Allele count*
      iii. *Minor peak vs. stutter product vs. artifact*
      iv. *Requirements for full vs partial vs uninterpretable*
   c) *Uninterpretable*

E. Documentation for Case record
   1. Electropherograms
   2. Project lists
   3. Reasons for re-injections
   4. Second read of raw data
   5. Profile comparison tool

F. Troubleshooting
   1. Identifying problems with data
   2. Determining causes of problems
      a) *Inhibition*
      b) *Degradation*
      c) *Offscale data*
      d) *Amplification template input*
   3. Determining solutions to problems

2.2 Required Readings

A. Butler John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch. 6.

B. Butler, John M. 2015. Advanced Topics in Forensic DNA Typing: Interpretation. Elsevier, Inc. Ch. 1-5, Ch. 6 pp 129-136, Ch. 8.

C. DNA Standard Operating Procedures, Texas Department of Public Safety Crime Laboratory (most recent version).
   1. Facilities;
   2. Analytical Controls;
   3. Response to Quality Issues;
   4. Case Documentation;
   5. Autosomal STR Data Evaluation and Profile Characterization Guidelines;
   6. Forms/Worksheets (as appropriate).

D. DNA System/Laboratory Validations (including local laboratory's CE implementation validation, sensitivity data study and threshold determination studies), Texas Department of Public Safety Crime Laboratory.

E. Any local policies as applicable.

F. DNA Fragment Analysis by Capillary Electrophoresis User Guide. Life Technologies (most recent/applicable version), Chapters 1, 2, 5, and 11.

G. Genemapper ID-X Software Reference Guide. Life Technologies (most recent/applicable version).

H. Investigator 24plex QS Handbook. Qiagen (most recent version).

I. Bregu, J, Conklin, D, Coronado, E, Terrill, M, Cotton, R and Grgicak, C. "Analytical Thresholds and Sensitivity: Establishing RFU Thresholds for Forensic DNA Analysis". Journal of Forensic Sciences. 2013. 58. 1. 2013. 120-129.

J. Scientific Working Group on DNA Analysis Methods (SWGDAM). SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. www.swgdam.org (most recent version) Section 1.

K. Gill, P., et al (2015) Genotyping and interpretation of STR-DNA: Low template, mixtures and database matches – Twenty years of research and development. FSI:Genetics, 18, 100-117.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the autosomal or Y-STR method described in the reading.

A. Scientific Working Group on DNA Analysis Methods (SWGDAM). SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing by Forensic DNA Testing Laboratories. www.swgdam.org. (most recent version) Sections 1 - 6. (**optional Yfiler or Y23**)

B. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). (**optional Yfiler or Y23)**

   1. Report Writing Guidelines;
   2. Y-STR Interpretation Guidelines;
   3. Y-STR Statistical Interpretation;
   4. Forms/Worksheets (as appropriate).

C. AmpF*l*STR MiniFiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (**optional Minifiler**)

D. AmpF*l*STR Yfiler PCR Amplification Kit User Guide. Life Technologies (most recent version). (**optional Yfiler**)

E. PowerPlex Y23 System Technical Manual. Promega (most recent version). (**optional Y23**)

3 Practice

3.1 Safety

A. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

B. Formamide is harmful if absorbed through the skin and is considered an irritant. Polymers are considered irritants.

3.2 Standards, Controls, Reagent Preparation

Standards and Controls

A. The appropriate amplification controls, the in-lane size standard, and a ladder must be included on each run.

B. The ladder and in-lane size standard are necessary for genotyping.

C. The appropriate amplification controls, the in-lane size standard, and the ladder ensure that the instrument is working properly and analyzing data appropriately.

D. Completion of required forms and proper documentation – refer to DNA SOP.

3.3 Equipment

A. For CE operation observation:

- Genetic Analyzer (Instrument, computer and appropriate software)
- Genetic Analyzer capillary arrays
- Genetic Analyzer septa/plates
- Genetic Analyzer buffer vials/reservoirs and septa
- Freezer or ice bath
- Heat block or thermal cycler
- Internal lane size standard appropriate for amplification kit type used
- Genetic analyzer buffer
- Matrix standard kit appropriate for amplification kit type used
- Hi-DI formamide
- POP-4 polymer
- Vortex
- Microcentrifuge tubes – 1.5 mL
- Pipettes
- Pipette tips
- Decapper
- Plate centrifuge or salad spinner
- Plate retainer and clip

B. For Data Analysis:

Computer with DNA analysis software

3.4 Observed Performance

A. Trainer will discuss capillary electrophoresis and the types of instruments used in the laboratory.

B. Trainee should observe the operation of capillary electrophoresis instrumentation for the collection of raw data including sample set up, sample run on instrument, and post run procedures (**optional**).

C. Trainer will demonstrate proper analysis of raw data using DNA analysis software including application of analytical threshold, documentation of run information, labeling of artifacts, and indicating reasons an injection was not used.

D. Trainer will demonstrate application of a stochastic threshold to a profile and how this application is used to determine if the profile is partial or full.

E. Trainer will demonstrate how to determine if a profile is a mixture or a single source profile.

F. Trainer will demonstrate a second read of the raw data.

3.5 Independent Exercises

A. Trainee will analyze raw data from previously amplified samples.

B. Trainee will troubleshoot problems using the data and suggest/implement ways to remedy these problems.

C. Dilution Samples

1. Obtain 3 DNA extracts previously quantified at approximately 0.5-1 ng/µL. Prepare 3 two-fold serial dilutions from each extract for a total of 12 samples.

   a) *Amplify the samples using the current validated autosomal amplification kit as outlined in the* Manual Amplification *module and run them on the CE instrument as outlined in the* Capillary Electrophoresis Instrument Operation and Troubleshooting *module to obtain raw data files. Samples may be injected for all validated injection times for the laboratory's instrument(s).*

   b) *Alternatively, the trainer may provide the trainee with raw data files from 3 two-fold serial dilutions of 3 DNA extracts amplified with the current validated autosomal STR amplification kit. Raw data files may be provided for each validated injection time for the laboratory's instrument(s).*

2. Analyze the samples at the validated analytical and stochastic thresholds established by each laboratory as outlined in the DNA SOP chapter: Autosomal STR Data Evaluation and Profile Characterization Guidelines.

3. Results including partial profiles, stochastic effects, effect of injection times, and the necessity for additional injections will be reviewed and discussed by the trainer and trainee. Observations and trends noted in the data should be documented by the trainee in their training notebook. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer.

4. If qualification in mini-STR interpretation is sought, the exercise from above will be completed using data from samples amplified with the current validated mini-STR kit.

D. For each amplification kit in which qualification is sought, trainee will be given data from a variety of single source and mixture profiles including partial profile data. Using the guidelines for characterization as outlined in the DNA SOP for autosomal and/or Y-STR interpretation, the trainee will characterize each profile as to partial or full and single source or mixture.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will analyze raw data from a minimum of 10 competency samples for each amplification kit in which qualification is sought. Analysis must include characterization of the profile as partial or full and single source or mixture, as well as labeling of any artifacts present in the data.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent analysis of DNA casework samples.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-03 MANUAL MIXTURE INTERPRETATION, DNA COMPARISON, AND MATCH STATISTICS

Purpose Provide experience making DNA comparisons and interpreting mixtures of varying concentrations and mixture proportions. Familiarize the analyst with the use of statistics in manual mixture interpretation of autosomal and Y-STRs. Discuss basic population genetics.

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived), Data Analysis and Sample Characterization module

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How data interpretation and comparisons are performed.

B. The importance of DNA quantity and how it affects sample typing results.

C. The criteria for determining number of contributors.

D. How to perform mixture interpretation and the factors that can make mixture interpretation difficult.

E. How to calculate mixture ratios and peak height ratios to determine relative contributions of donors in a mixture.

F. How to apply the 60% rule to determine the major component of a mixed source in Y-STR (Yfiler or Y23) profiles.

G. Basic principles of population statistics including Hardy-Weinberg equilibrium, Linkage equilibrium, population substructure and the ceiling principle.

H. The statistics currently and previously used by the Texas DPS Crime Laboratory Services including: random match probability, combined probability of inclusion, modified random match probability, likelihood ratios, and the counting method for Y-STR.

I. The databases used by the Texas DPS Crime Laboratory Services.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Properly apply a stochastic threshold to mixture profiles.

B. Determine the number of contributors.

C. Assess data through use of stochastic thresholds, degradation curves, and interlocus rule.

D. Resolve major, minor, and mixed major components, use a known contributor to deduce a foreign contributor, and select suitable loci to use for inclusion statistics.

E. Make comparisons of known profiles to evidentiary-type profiles.

F. Conclude which individuals would be excluded or included in mixed samples.

G. Manually calculate significance estimates for single source samples using RMP and mixed source samples using CPI.

H. Learn to use the online YHRD for Y population calculations (**optional**).

1.3 Considerations

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.

A. Autosomal DNA Profiles

1. 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 Combined DNA Index System (CODIS) entry.
2. Once an association has been identified and an individual included as a possible source of evidentiary material, the significance of that association is estimated to allow investigators, the legal sector, and ultimately a jury of lay persons to place the appropriate emphasis on the conclusion.
3. Significance estimation will be expressed as an inverse probability of inclusion (random match probability and combined probability of inclusion) or likelihood ratios.
4. The latest version of the FBI's *Popstats* software or other validated software will be configured and installed to calculate single source and mixed source significance estimates. Population data will be taken from the NIST database published in FSI:Genetics in 2013.

B. Y-STR DNA Profiles

1. The basis for the haplotype frequency estimation is the counting method. According to the Scientific Working Group on DNA Analysis Methods (SWGDAM), reporting a haplotype count without a confidence interval is acceptable as a factual statement regarding observations in the database.
2. Our laboratory system will use the Y-chromosome Haplotype Reference Database (YHRD), and will report the number of times a haplotype has been seen in the total U.S. database. Caucasian, African American, Hispanic, Asian, and Native American databases are also available.
3. The profile count will be reported as a statement regarding observations in the database and expressed as numbers truncated to the last significant digits.
4. The Y-chromosome Haplotype Reference Database (YHRD) can be located on the internet at the following address: http://yhrd.org. This database was designed for use in estimating Y-STR haplotype population frequencies for forensic casework purposes.

1.4 Position Statement for Texas DPS Crime Laboratory System – DNA Discipline

As DPS analysis methods evolved from RFLP, DQ alpha, and D1S80 into multiplex kits, our ability to interpret mixtures of DNA increased. We were able to amplify multiple loci simultaneously and found that we could obtain profiles of higher quality with less DNA template input because of the sensitivity of the kits. We continued to apply knowledge that we gained from two-person mixtures to more complex mixtures (three or more person) and partial profiles, which was consistent with other laboratories in the country. We validated more sensitive amplification kits and instruments. We continued to evaluate and revise DNA interpretation guidelines.

From the implementation of DNA analysis until 2015, our statistical approach for mixtures (Combined Probability of Inclusion) had been based on recommendations made by the FBI DNA

Advisory Board. (Statistical and Population Genetics Issues Affecting the Databases(s). For Sci Comm. 2000. 2(3). U.S. Department of Justice, Federal Bureau of Investigation).

Texas DPS DNA Labs moved to implementation of a dual interpretation threshold consisting of an analytical threshold (AT) and a stochastic threshold (ST) effective August 10, 2015. This decision was reached after careful consideration of several factors. These factors included but were not limited to:

- Recommendations put forth in the 2010 SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories
- Inquiries by the legal community regarding current statistical interpretations in court
- The progression of the Forensic DNA community away from the binary statistical interpretation model
- External assessment

It was recognized that CPI statistical application does not make efficient use of available information given in complex mixtures and partial profiles. Due to the sensitivity of new amplification kits, a different statistical approach was needed to limit the inclusionary weight of the evidence, while maximizing the exculpatory information in the profile. The DNA Advisory Board acknowledges the best approach to handling these types of data was to incorporate the use of probabilistic genotyping software (see the module *STRmix Mixture Deconvolution Software and Likelihood Ratios*).

A dual threshold approach is also available for use in casework data interpretation. This module serves to educate the analyst on the use of the methods used in manual interpretation using dual thresholds.

2 Training Outline

2.1 Lesson Plan

A. Interpretation
   1. Thresholds
      a) Analytical
      b) Stochastic
   2. Conclusive (suitable) loci
   3. Inconclusive (unsuitable) loci
   4. Bias

B. Mixture Studies
   1. Assigning number of contributors
   2. Classification as intimate, indigenous, and environmental based on sample origin
   3. Major, minor, foreign, and mixed major component identification
   4. Mixture Ratios and Proportions
   5. Balance across loci – applying the interlocus rule
   6. Indistinguishable mixture

7. Inconclusive minor component of a distinguishable mixture
8. Uninterpretable profile

C. Comparisons
1. Inclusions
2. Exclusions
3. Inconclusive
4. Uninterpretable
5. No results

D. Overview of population genetics
1. PCR-based systems
   a) DQ-Alpha
   b) D1S80
   c) STR
2. Hardy-Weinberg (HW) equilibrium
   a) Randomly Mating Populations
   b) Linkage equilibrium (LE)
   c) Population structure
   d) Subpopulations
3. Statistical considerations
   a) The reference database
   b) Restricted (binary approach)
   c) Match Probability (random match)
   d) Modified RMP
   e) Combined Probability of Inclusion (CPI)
   f) Identification of suspect by database
   g) Uniqueness
4. The Ceiling Principles
5. DNA in the courts

E. The 1992 NRC Report
1. The validity of DNA typing
2. The use of DNA for exclusion
3. Changes since the 1992 report
4. Paternity testing

F. Conclusions and Recommendations of the NRC

G. Use of Binary Statistics by the Texas DPS Crime Laboratory Services

1. Autosomal

a) Significance estimation

i. Single source significance calculation (RMP)

ii. Mixed source significance calculation (RMP and CPI)

iii. Use of 2p-p2 for consideration of drop-out

iv. Use of obligate alleles

v. Minimum and Null Allele frequencies

vi. Off-ladder alleles

vii. Software

- ***Popstats***
- ***RMP tool***

b) Population databases

i. Texas/FBI population database

ii. NIST population database

iii. Database errors

c) Population frequency tables

2. Y-STRs – Online database YHRD (http://yhrd.org)

2.2 Required Readings

Readings are selected based on if the trainee seeks qualification for mini-STR or Y-STR methods. It is required that all trainees undertaking this module qualify on interpretation with the current validated autosomal STR amplification kit at a minimum. The readings in this section are required for all trainees.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Butler, John M. 2015. Advanced Topics in Forensic DNA Typing: Interpretation. Elsevier, Inc. Ch. 6 pp 136-158, Ch. 7, 9-13, Appendix 4.

B. Local laboratory's CE implementation validation mixture study data.

C. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

1. Validation;
2. Autosomal STR Data Evaluation and Profile Characterization Guidelines;
3. Autosomal STR Interpretation Guidelines;
4. Interpretation Flowcharts;
5. Statistical Significance Estimation;
6. Texas/FBI STR Population Database;
7. NIST STR Population Database;

8. Report Writing Guidelines;
9. Forms/Worksheets (as appropriate).

D. Any local policies as applicable.

E. Committee on DNA Technology in Forensic Science: an Update. II. National Research Council (U.S.). Commission on DNA Technology in Forensic Science: an Update. The Evaluation of Forensic DNA Evidence. National Academy Press. Washington, D.C. 1996. pp 21-23 and 26-28, Chapter 4, p.89-124, Chapter 5, p.125-162.

F. Scientific Working Group on DNA Analysis Methods (SWGDAM). SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. www.swgdam.org (most recent version) Sections 2 and 3.

G. Dror, I. and Hampikian, G. Subjectivity and Bias in Forensic Mixture Interpretation. 2011. Science and Justice (51). pp. 204-208.

H. Boston University Biomedical Forensic Sciences DNA Mixtures Online Training (relevant sections). Available at www.bu.edu.dnamixtures/8/. Username and password will be provided by trainer.

I. Budowle, B., Onorato, A. J., Callaghan, T. F., Manna, A. D., Gross, A. M., Guerrieri, R. A., Luttman, J. C. and McClure, D. L. (2009), Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework. Journal of Forensic Sciences, 54: 810–821.

J. Biedermann, A., et al (2012) Inference about the number of contributors to a DNA mixture: comparative analyses of Bayesian network approach and the maximum allele count method. FSI: Genetics, 6 689-696.

K. Buckleton, J.S., et al (2007) Towards understanding the effect of uncertainty in the number of contributors to DNA stains. FSI Genetics, 1, 20-28.

L. Pfeifer, C., et al (2012) Comparison of different interpretation strategies for low template DNA mixtures. FSI: Genetics, 6, 716-722.

M. Gill, P. et al. DNA Commission of the ISFG: Recommendations on the Interpretations of Mixtures. 2006. Forensic Science International (160). pp 90-101.

N. Hill, C.R., Duewer, D.L., Kline, M.C., Coble, M.D., Butler, J.M. (2013) U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 7: e82-e83 (Supplemental Material Table 2).

O. Steffen C.R., Coble M.D, Gettings K.B., Vallone P.M. (2017) Corrigendum to 'U.S. Population Data for 29 Autosomal STR Loci' [Forensic Sci. Int. Genet. 7(2013) e82-e83] Forensic Sci. Int. Genet. 31: e36-e40.

P. Balding, D. and Buckleton, J. "Interpreting low template DNA profiles" Forensic Science International: Genetics. 2009. 4. 1-10.

Q. Bieber, F., et al (2016). Evaluation of forensic DNA mixture evidence: protocol for evaluation, interpretation, and statistical calculations using the combined probability of inclusion. BMC Genetics (2016) 17:125.

R. Rommel, M. DPS Internal Training: Manual Mixture Interpretation SOP Changes. 2016.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the autosomal or Y-STR method described in the reading.

A. Scientific Working Group on DNA Analysis Methods (SWGDAM). SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing by Forensic DNA Testing Laboratories. www.swgdam.org. (most recent version) Sections 7 -10.

B. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

   1. Report Writing Guidelines;
   2. Y-STR Interpretation Guidelines;
   3. Y-STR Statistical Interpretation;
   4. Forms/Worksheets (as appropriate).

3 Practice

3.1 Safety

A. Wear lab coat and gloves when working in the laboratory. A mask may be worn to further protect against contamination.

B. Safety glasses may be required during some procedures. Read SDS's for chemicals used in laboratory.

C. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

3.2 Standards, Controls, and Reagent Preparation

Controls

A. It is necessary to run the appropriate amplification controls, the in-lane size standard, as well as a ladder with each instrument run. This ensures the instrument is working properly and analyzing data appropriately.

B. Completion of required forms and examples of proper documentation.

3.3 Equipment

See *Manual Amplification* and *Capillary Electrophoresis Instrument Operation and Troubleshooting* modules for equipment lists.

3.4 Observed Performance

A. For autosomal STR interpretation:

   1. The trainer will demonstrate manual mixture deconvolution on at least one mixture profile for the following mixture types: major/minor, foreign component, mixed major, indistinguishable.
   2. Trainer will demonstrate use of the Deconvolution Tool listed in the DNA SOP.
   3. The trainer will demonstrate the manual calculation of the significance estimate of at least one single source profile (RMP) and one mixed source profile (CPI).

4. The trainer will demonstrate the *Popstats* software and/or other validated software such as the RMP tool. The trainer will be present at all times when the trainee is using the *Popstats* software.

B. If qualification is sought in mini-STR interpretation: the trainer will demonstrate manual mixture deconvolution on at least one mixture profile using data amplified with the current validated mini-STR amplification kit.

C. If qualification is sought in Y-STR interpretation:

1. The trainer will demonstrate how to determine if a mixture is distinguishable or indistinguishable on at least one mixture profile using data amplified with a current validated Y-STR amplification kit.

2. The trainer will demonstrate the use of the online database YHRD for Y population calculations (http://yhrd.org)

3.5 Independent Exercises

A. For autosomal STR interpretation:

1. Mixture Samples

a) Refer to the information on mixture interpretation in the DNA SOP (Autosomal STR Data Evaluation and Profile Characterization Guidelines and Manual Autosomal STR Interpretation Guidelines).

b) Obtain four DNA extracts previously quantified at approximately 0.5-1 ng/µL: three from females (donors A, C, D) and one from a male (donor B).

c) Mix at the following ratios to obtain the appropriate amount of input DNA template. Note that the male donor (B) should be the lowest level contributor when appropriate:

1 :1	ratio of A:B	5 :5 :1	ratio of A:C:B
4 :1	ratio of A:B	*10* :5 :1	ratio of A:C:B
10 :1	ratio of A:B	*10* :*10*:1	ratio of A:C:B
20 :1	ratio of A:B	1:1 :1 :1	ratio of A:B:C:D
1 : 1 :1	ratio of A:B:C	*10*:5 :5 :1	ratio of A:C:D:B
10 : 1 :1	ratio of A:B:C		

d) Amplify the samples using the currently validated autosomal amplification kit as outlined in the Manual Amplification *module and run them on the CE instrument as outlined in the* Capillary Electrophoresis Instrument Operation and Troubleshooting *module to obtain raw data files. Samples may be injected for all validated injection times for the laboratory's instrument(s).*

e) Alternatively, the trainer may provide the trainee with raw data files from the mixture ratios listed above that have been amplified with the current validated autosomal STR amplification kit. Raw data files may be provided for each validated injection time for the laboratory's instrument(s).

f) The trainee must review these profiles and make observations regarding partial profiles, stochastic effects, number of observed contributors versus number of actual contributors, effect of injection times, and the necessity for additional injections.

g) *Results will be reviewed and discussed by the trainer and trainee. Observations and trends noted in the data should be documented by the trainee in their training notebook. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer.*

h) *If qualification in mini-STR interpretation is sought, the exercise from above will be completed using data from samples amplified with the current validated mini-STR kit.*

2. Manual Mixture Deconvolution and Comparison Exercise

a) *Read the information on mixture interpretation in DNA SOP (Autosomal STR Data Evaluation and Profile Characterization Guidelines and Manual Autosomal STR Interpretation Guidelines).*

b) *The trainee will be given at least 10 sets of DNA data for the current validated autosomal STR amplification kit. Using the DNA SOP STR Interpretation Guidelines, the analyst should assign a number of contributors, classify the profile as intimate, indigenous, or environmental, characterize the profile as full or partial, determine if a major component is present, calculate the average mixture ratio, and determine which individuals would be excluded or included for each set of data. Statistical calculations (using software) will be performed to determine the significance of the association, as applicable. The trainer may work through one of these profiles with the trainee to meet supervised performance requirements mentioned above.*

c) *The Technical Leader, trainer, or their designee will assess the trainee's understanding of manual mixture deconvolution and may assign additional exercises as they feel appropriate.*

d) *All completed documentation from these exercises will be included and retained in the trainee's training notebook.*

e) *If qualification in mini-STR interpretation is sought, the exercise from above will be completed using data from samples amplified with the current validated mini-STR kit.*

3. Manual Statistic Calculation

a) *The trainee will manually calculate the significance estimate for one single source profile using RMP and one mixed source profile using CPI using the NRC II 4.1 formulae and population frequency tables.*

b) *These profiles may be full or partial, but should contain at least one heterozygous locus, one homozygous locus, and one locus exhibiting possible dropout.*

B. If qualification is sought in Y-STR interpretation:

1. Y-STR comparison exercise

The trainee will be given 5 sets of mixture data generated from each validated Y-STR amplification kit on which qualification is sought. The trainee will use these data sets to determine if the mixture is distinguishable or indistinguishable and what comparison (if any) can be made.

2. Y-STR statistic calculation

The trainee will apply the online database YHRD to generate statistics on 5 sets of Y-STR data for each validated Y-STR amplification kit on which qualification is sought.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainee will be given at least 5 sets of DNA data for the current validated autosomal STR amplification kit. Based on their understanding of the DNA SOP STR Interpretation Guidelines, the trainee should assign a number of contributors, characterize the profile as full or partial, determine if a major component is present, calculate the average mixture ratio, and determine which individuals would be excluded or included for each set of data. Statistical calculations (using software) will be done to determine the significance of the association as applicable.

B. If qualification in mini-STR interpretation is sought, the trainee will be given at least 5 sets of DNA data for the current validated mini-STR amplification kit. Based on their understanding of the DNA SOP STR Interpretation Guidelines, the trainee should assign a number of contributors, characterize the profile as full or partial, determine if a major component is present, calculate the average mixture ratio, and determine which individuals would be excluded or included for each set of data. Statistical calculations (using software) will be done to determine the significance of the association as applicable.

C. If qualification in Y-STR interpretation is sought, the trainee will be given at least 5 sets of DNA data for each currently validated Y-STR kit for which qualification is sought. Based on their understanding of the DNA SOP Y-STR Interpretation Guidelines, the trainee should assign a number of contributors, characterize the profile as full or partial, determine if any mixtures are distinguishable or indistinguishable, and determine which individuals would be excluded or included for each set of data. The database will be used to calculate a statistic (counting method) used to determine the significance of the association as applicable.

D. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-04 STRMIX MIXTURE DECONVOLUTION SOFTWARE AND LIKELIHOOD RATIOS

Purpose To provide experience in interpreting single source and mixtures of varying concentrations utilizing STRmix probabilistic genotyping software and likelihood ratios.

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived), Data Analysis and Sample Characterization Module, Manual Mixture Interpretation, DNA Comparison, and Match Statistics module.

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How interpreting profiles from minimal stains with limited quantities of DNA, multiple donors resulting in complex mixtures, and/or low level data is critical for inclusion/exclusion purposes and Combined DNA Index System (CODIS) entry.

B. The challenges and limitations involved when working with small/dilute samples.

C. How DNA quantity of a sample affects STRmix results.

D. How varying number of contributors to a sample affect the STRmix results.

E. Likelihood Ratios and how the ability to construct competing likelihood ratio hypotheses and report the resultant likelihood ratio is paramount in expressing the importance of DNA evidence as it pertained to criminal legal proceedings.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Analyze and interpret the dilution and mixture samples using the STRmix software.

B. Construct appropriate likelihood ratios, including when to condition the likelihood ratio on the presence of an individual.

1.3 Considerations

A. Background

Due to the sensitivity of new amplification kits, a different statistical approach was needed to limit the inclusionary weight of the evidence, while maximizing the exculpatory information in the profile. The DNA Advisory Board acknowledges the best approach to handling these types of data was to incorporate the use of probabilistic genotyping software.

B. Reason for Manual Profile Evaluation

1. A manual evaluation by the analyst is crucial in determining that the software runs appropriately. For example, minor mistakes in data entry can lead to false exclusions of individuals by resulting in a likelihood ratio (LR) of zero.

2. While the software is able to consider the multitude of possible genotypes and assign weights from the most likely to the least likely in a manner that would be time prohibitive to do manually, it is not a substitute for a manual evaluation of the data. The possibility for adventitious support for exclusions or inclusions exists

when using the software. Additionally, inconclusive comparisons may result from data that is actually exclusionary.

3. During manual evaluation of the data, the analyst must look at the electropherogram to assess if the STRmix output is supported by what is seen in the electropherogram. Analysts must assess if the mixture ratios, likelihood ratio, and resulting conclusion are consistent between STRmix output and the electropherogram.

2 Training Outline

2.1 Lesson Plan

A. Math Review
   1. Binomials
   2. Factorization
   3. Squaring numbers
   4. Slope
   5. Degradation Curve
   6. Algebraic Notation
   7. Log
   8. Distributions
   9. Hardy-Weinberg
   10. Probability
   11. Sample Point
   12. Events
   13. Ratios

B. Interpretation Models
   1. Semi-Continuous
   2. Continuous

C. Likelihood Ratios (LR)
   1. LR Principles
   2. Bayes' Theorem
   3. Hierarchy of Propositions
   4. Forming Propositions
   5. Reporting LR Statements
   6. LR Testimony – Fallacies
   7. LR and STRmix Software
      a) Balding Nichols Equation
      b) Highest Posterior Density (HPD)

D. STRmix Software
   1. Model Maker
   2. Import Settings
   3. Software Settings
   4. Number of Contributors
   5. Run Diagnostics
   6. Longest Uninterrupted Sequence (LUS) Theory
   7. Markov Chain Monte Carlo (MCMC)
   8. Metropolis-Hastings

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. Texas DPS System STRmix validations for any currently used autosomal and mini-STR amplification kits.

B. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   1. Autosomal STR Data Evaluation and Profile Characterization Guidelines
   2. Manual Autosomal STR Interpretation Guidelines;
   3. STRmix Autosomal Interpretation Guidelines;
   4. Report Writing Guidelines.

C. Any local policies as applicable.

D. Institute of Environmental Science and Research Limited. STRmix Users Manual (Most recent version) (Sections 1-5).

E. Institute of Environmental Science and Research Limited. STRmix v2.3 Users Manual-Texas DPS. (Section 12).

F. Institute of Environmental Science and Research Limited. STRmix v2.4 Users Manual – Texas DPS. (Sections 2.16 – 2.20 and 5).

G. Budowle, B. et al. (2009). Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework. Journal Forensic Science, 54, 810-821.

H. Cooper, S., McGovern, C., Bright, J.-A., Taylor, D., and Buckleton, J. (2015). Investigating a common approach to DNA profile interpretation using probabilistic software. Forensic Science International: Genetics, 16, 121-131.

I. Kelly, H., Bright, J.-A., Buckleton, J. S., and Curran, J. M. (2014). A comparison of statistical models for the analysis of complex forensic DNA profiles. Science and Justice, 54, 66-70.

J. STRmix Niagara County NY Decision

K. DPS 4 day STRmix/LR training, Austin, 2016. 4 DVD set.

L. Taylor, D. (2014). Using continuous DNA interpretation methods to revisit likelihood ratio behavior. FSI: Genetics, 11 (2014), 144-153.

M. Taylor, D. (2015). Is STRmix Reliable? http://support.strmix.com/support/login

N. Bright, J. et al. (2015). The variability in likelihood ratios due to different mechanisms. FSI: Genetics, 14 (2015), 187-190.

O. Scientific Working Group on DNA Analysis Methods (SWGDAM). SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories. www.swgdam.org (most recent version).

3 Practice

3.1 Safety

A. Wear lab coat and gloves when working in the laboratory. A mask may be worn to further protect against contamination.

B. Safety glasses may be required during some procedures.

C. Read SDS's for chemicals used in laboratory.

D. Appropriate personal protective equipment must be worn during reagent preparation and use. Clothing may protect unbroken skin; broken skin must be covered.

3.2 Standards, Controls, and Reagent Preparation

Controls

A. It is necessary to run the appropriate amplification controls, the in-lane size standard, as well as a ladder with each instrument run. This ensures the instrument is working properly and analyzing data appropriately.

B. Completion of required forms and proper documentation.

3.3 Equipment

See *Manual Amplification* and *Capillary Electrophoresis Instrument Operation and Troubleshooting* modules for equipment lists.

3.4 Independent Exercises

A. Dilution Samples

1. Using the data from the *Data Analysis and Sample Characterization* module, the trainee will deconvolute all samples through the STRmix software, construct appropriate likelihood ratios, report the resultant LR value and state its implication for inclusion, exclusion or inconclusive.

2. Results will be reviewed and discussed by the trainer and trainee. Observations and trends noted in the data should be documented by the trainee in their training notebook. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer.

B. Mixture Samples

Refer to the information on mixture interpretation in the DNA SOP (*Manual Autosomal STR Interpretation Guidelines* and *STRmix* chapters).

1. Using the data from the *Manual Mixture Interpretation, DNA Comparison, and Match Statistics* module along with electronic data for the known profiles used to

develop the data in this module and several profiles that are known exclusions, trainee will deconvolute all samples through the STRmix software, construct likelihood ratios, condition the likelihood ratio when appropriate, demonstrate the use of co-contributors, compare the STRmix mixture ratios to the expected ratios, examine the diagnostics required by the SOP, and document the resultant likelihood ratio values.

2. The likelihood ratios and their implications for inclusion, exclusion, or inconclusiveness will be discussed with the trainer. Observations and trends noted in the data should be documented by the trainee in their training notebook. The trainee will repeat any samples not meeting the expected criteria or as deemed necessary by the trainer.

C. Manual statistic calculation:

The trainee will manually calculate a single source significance point estimate (LR) using the Balding-Nichols formulae, posterior mean allele frequency, and population frequency tables.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

B. The trainee will be given at least 5 scenarios with accompanying sets of data to be interpreted using the STRmix software. These 5 scenarios may be part of the written examination or assigned separately.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-05 CODIS

Purpose To inform and instruct the trainee about the uses and laws related to the COmbined DNA Index System. To teach the trainee how to deduce a profile that is eligible for CODIS upload.

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived), Data Analysis and Sample Characterization module, Manual Mixture Interpretation, DNA Comparison, and Match Statistics module, STRmix Mixture Deconvolution Software and Likelihood Ratios module

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. What a database is and how to distinguish between different databases, and different indexes (LDIS, SDIS, NDIS).

B. The background and state and federal laws associated with the collection of samples for CODIS.

1.2 Practical

Upon completion of this training, the trainee will be able to:

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

B. Apply skills learned in the Manual Mixture Interpretation, DNA Comparison, and Match Statistics module to deduce profiles from mixtures that are eligible for entry into the CODIS database.

1.3 Considerations

"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." (Source: US Department of Justice, the FBI's DNA and Databasing Initiatives, October 2000.)

A. Terms

1. Known/assumed contributor: the person who could be expected in a mixture profile and whose profile may not be entered into CODIS, e.g. victim profile on vaginal swab, stain from victim's clothing or bedding, or consensual partner's profile on vaginal swab

2. Unknown contributor: the putative perpetrator whose profile may be entered into CODIS if it is a single source profile or once it has been deduced from a mixture profile

B. General CODIS entry guidelines

1. Single source/major component CODIS eligible profiles should be entered into CODIS.

2. It is inappropriate to use the suspect's known profile to deduce a profile for CODIS entry. It is not appropriate to use an evidentiary profile to deduce a profile for CODIS entry. Assumed contributors may be used to deduce a profile for CODIS entry.

3. Alleles unambiguously attributed to a victim or elimination sample shall not be entered into CODIS. Alleles not clearly attributed to a victim or elimination sample may be entered into CODIS after thorough evaluation of the profile.
4. Not all evidentiary profiles will be suitable for CODIS entry.
5. If any locus has only one or two alleles it may be more conservative to not enter alleles for that locus into CODIS if the unknown contributor is a minor contributor to the profile.
6. Caution must be used in applying these rules if allelic drop out/ drop in is suspected.
7. The procedure for manual mixture deconvolution may be used to deconvolute profiles for CODIS entry. Refer to the DNA SOP chapter: Manual Autosomal STR Interpretation Guidelines for manual mixture deconvolution procedures.

   Alternatively, STRmix may be used to deconvolute profiles for CODIS entry. Refer to the DNA SOP chapter: STRmix for procedures.
8. If it is not possible to determine all of the alleles being contributed by the unknown contributor due to the complexity of the mixture profile obtained, entering more than 2 alleles per locus is an option. These profiles must be marked as mixture profiles prior to CODIS entry.
   a) *Alleles that must have been contributed by the unknown contributor are referred to as required alleles.*
   b) *If an allele is not present in the known contributor's profile this may indicate that it is a required allele.*
   c) *Required alleles are designated with a "+" after the allele.*
   d) *Only one allele per locus may be designated as a required allele. If only one allele is entered at a locus there is no need to use the required allele designation.*
9. Any partial or mixture evidentiary profile shall be evaluated by a match estimation tool prior to entry.
   a) *A profile must be evaluated before being marked for upload using the Moderate Match Estimate (MME) threshold or the inverse Match Rarity Estimate (MRE).*
   b) *The MME is automatically calculated in the CODIS software once entered and saved. The inverse MRE can be calculated using the Match Estimation Tool in Popstats. Refer to the DNA SOP chapter: CODIS) for details.*

C. CODIS Specimen Categories (Indexes)

1. LDIS (Local DNA Index System)
   a) *Required number of loci for entry of autosomal profiles into LDIS is determined by local policy.*
   b) *Specimen categories existing in LDIS are determined by local policy. These categories are not uploaded and are searched only at LDIS.*
   c) *DNA Y-STR profiles are entered into a local Y-STR index at LDIS. Required number of loci for entry into the LDIS Y-STR index is a minimum of 10 from any Y-STR loci.*

2. SDIS (State DNA Index System) – required number of loci for entry of autosomal profiles into SDIS is a minimum of 7 from the original core and D2S1338 and D19S433 (not including Amelogenin). Y-STR data that is associated with an autosomal profile is uploaded to but not searched at SDIS.

   a) *SDIS Forensic Partial – this category contains single source profiles or fully deduced profiles originating from mixtures. The profiles have either locus or allelic information missing at any of the core loci and meet an MME or MRE value of 1.00E+5. There are no more than three alleles at any one locus in the profile.*

   b) *SDIS Forensic Mixture – this category contains profiles with DNA from more than one source. The profiles shall not have more than 4 alleles at any one locus. The profiles meet an MME or MRE value of 1.00E+5.*

   c) *Suspect Known – this category contains profiles developed from a suspect reference sample where locus or allelic information is missing at any of the following loci: TH01, D3S1358, vWA, D21S11, TPOX, D1S1656, D12S391, D10S1248, D22S1045, D19S433, D8S1179, D2S1338, D2S441, D18S51, FGA, D16S539, CSF1PO, D13S317, D5S818, and D7S820. This category also contains partial or complete profiles developed as alternate knowns for suspects.*

3. NDIS (National DNA Index System) - For autosomal profiles, NDIS requires that all core loci be attempted and will accept specimens with data for 8 of the original 13 core loci (not including Amelogenin) for searching. Amelogenin is accepted at NDIS although not searched. Y-STR data that is associated with an autosomal profile is not uploaded to or searched at NDIS at this time.

   a) *Forensic unknown - this category contains single source profiles or fully deduced profiles originating from mixtures having all core loci and shall not have more than 3 alleles at any one locus in the profile.*

   b) *Forensic Partial – this category contains single source profiles or fully deduced profiles originating from mixtures with 8 or more original core loci. The profiles have either locus or allelic information missing at any core loci and meet an MME or MRE value of 1.00E+7. There are no more than 3 alleles at any one locus in the profile. For example, profiles with required/obligate allele(s), locus indicator of partial (p), or profile indicator of partial "yes" must be entered into this index.*

   c) *Forensic Mixture – this category contains profiles with DNA from more than one source with 8 or more original core loci. The profiles shall not have more than 4 alleles at any one locus. The profiles meet an MME or MRE value of 1.00E+7.*

   d) *Forensic Targeted- this category is intended for Forensic Partial and Forensic Mixture specimens that do not meet a 1 in 10 million MME value. This category will only be searched against full single source profiles (Forensic Unknown, Convicted Offender, Arrestee, Detainee, and Legal). This category should be considered a specimen category of last resort for NDIS and specimens that meet MME should remain in the Forensic Partial or Forensic Mixture specimen category over placing them in the Forensic Targeted specimen category. Specimens in this category must have 8 or more original core loci with an MRE (stringency by locus) value of 1 in 10 million. The Forensic Targeted index is only searched "stringency by locus" at NDIS. Loci marked partial and loci with more than 2 alleles are searched at Moderate and all other loci are searched at High Stringency. Loci with the required allele designation do not get automatically searched at Moderate.*

e) Legal – this category includes eligible profiles from suspect reference samples with complete information at all 20 core loci. If suspect profiles do not meet the requirement for this index, they will be entered into the Suspect Known index.

2 Training Outline

2.1 Lesson Plan

A. COmbined DNA Index System (CODIS) hierarchy

1. NDIS-National DNA Index System
2. SDIS-State DNA Index System
3. LDIS-Local DNA Index System

B. Indexes/Specimen Categories

1. Forensic Unknown
2. Suspect
3. Missing Persons
4. Relatives of Missing Persons
5. Unidentified Remains
6. Arrestee
7. Victim
8. Detainee
9. Elimination
10. Mixture
11. Legal
12. SDIS Forensic Partial
13. SDIS Forensic Mixture

C. CODIS casework database

1. Specimen Identification
2. Forensic Unknowns
3. Suspect Knowns
4. Forensic Mixtures-resolving mixtures for CODIS entry
   - *a) Peak height ratio calculations*
   - *b) Major/minor component*
5. Match Estimation
   - *a) Moderate Match Estimate (MME)*
   - *b) Inverse Match Rarity Estimate (MRE)*
6. Specimen Sample Types
   - *a) Forensic Unknown*

b) Forensic Mixture vs SDIS Forensic Mixture,

c) Forensic Partial vs SDIS Forensic Partial

d) Legal vs Suspect

D. CODIS reporting

E. State CODIS Laboratory

1. Forensic Hits

a) Case to case

b) Case to suspect

2. Offender Hits

3. Legal Hits

4. Autosearching

5. Uploads

6. Backups

F. Expansion of required core loci from 13 to 20.

2.2 Required Readings

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

1. Outsourcing;

2. Manual Autosomal STR Interpretation Guidelines;

3. Report Writing Guidelines;

4. CODIS;

5. Y-STR Interpretation Guidelines;

6. STRmix;

7. Forms/Worksheets (as appropriate).

B. Li, R. Forensic Biology: Identification and DNA Analysis of Biological Evidence. 2008. CRC Press. Sections 22.2-22.4.

C. Butler, John M. 2012. Advanced Topics in Forensic DNA Typing: Methodology. Elsevier, Inc. Ch.8 and 9.

D. Hares, D. Expanding the CODIS core loci in the United States. Forensic Science International: Genetics 2012. 6, pp e52-e54.

E. NDIS Procedures most recent update, see Criminal Justice Information Services – Shared Enterprise Network (CJIS-SEN) website.

1. NDIS Laboratories

2. NDIS Acceptance Standards

3. NDIS Searches
4. Confirmation and Hit Dispositioning
5. NDIS Security Requirements

F. CODIS Administrators Handbook (Appendix D: Sample NDIS Eligibility Scenarios). (most recent update, see CJIS-SEN website.)

G. CODIS 8.0 Computer Based Training (most recent update, see CJIS-SEN website).

1. How to use the CBT
2. CODIS 8.0 Overview
3. CODIS 8.0 STR Data Entry/Y-STR DNA Data Entry
4. CODIS 8.0 Specimen Manager
5. CODIS 8.0 Options-Popstats
6. CODIS 8.0 Stringency
7. CODIS 8.0 Required Alleles
8. CODIS 8.0 Popstats FSS
9. CODIS 8.0 Popstats Mixtures
10. CODIS 8.0 Popstats Match Estimation

H. NDIS Forensic Index Decision Tree (most recent update, see CJIS-SEN website).

I. Any local policies/validations as applicable.

3 Practice

3.1 Safety

None

3.2 Standards, Controls, and Reagent Preparation

None

3.3 Equipment

None

3.4 Observed Performance

A. The trainer will demonstrate which single source or deduced profiles are eligible for upload into the CODIS database and to which level.

B. The trainer will demonstrate how to deduce CODIS profiles from mixtures using manual mixture deconvolution and STRmix procedures.

C. The trainer will discuss and demonstrate how to calculate MME/MRE.

3.5 Independent Exercises

A. The trainee will be given at least 5 data sets including electropherogram(s) with stutter filters on, accompanying case-like scenarios and optional reference profiles for the trainee to manually deduce CODIS profiles from mixtures, and fill out the appropriate paperwork for the profile to be entered.

B. The trainee will be given at least 5 data sets including STRmix results files, corresponding electropherograms, accompanying case-like scenarios and optional reference profiles for the trainee to utilize the results to deduce CODIS profiles from mixtures, manually verify the deduced profile and fill out the appropriate paperwork for the profile to be entered.

C. The data sets provided as part of the 20 cases required for the *DNA Report Writing and Case Review* module or the data sets from the *Manual Mixture Interpretation, DNA Comparison, and Match Statistics* and *STRmix Mixture Deconvolution Software and Likelihood Ratios* modules may be used to fulfill this requirement.

Note: If casework data sets are used, all case information including case numbers, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.

4 Assessment

4.1 Competency and Qualifying Examination

A. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee.

B. The trainee must successfully complete the individual module quizzes for the Computer Based Training in the Learning Management System of the most recent version of CODIS.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-06 DNA REPORT WRITING AND TECHNICAL REVIEW

Purpose Provide trainee with guidelines for DNA analysis casework report writing and for technical and administrative case reviews.

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived), Data Analysis and Sample Characterization module, Manual Mixture Interpretation, DNA Comparison, and Match Statistics Module, STRmix Mixture Deconvolution Software and Likelihood Ratios module, CODIS module

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How the laboratory report is used to communicate to its reader the analytical results, conclusions of the analyst, and statistical statements supporting that conclusion. It conveys the essence of what would be said if the analyst were asked to give an opinion in court.

B. How the report is the method through which the analyst is able to state what the evidence means and also what it does not mean. It is highly desirable that the report be able to "stand alone" since decisions may be made by police officers, attorneys and the courts, based on the report, with no contact with the analyst.

C. How a thorough review of each case record is performed to ensure that conclusions and supporting data are reasonable and within the constraints of scientific knowledge.

D. The two separate review components to the case review process.

1. Technical Review
   - *a) How to conduct an evaluation of documentation to check for consistency, accuracy, and completeness.*
   - *b) How to conduct an evaluation of reports, notes, data and other documents to ensure an appropriate and sufficient basis for the scientific conclusions.*
   - *c) The requirements for personnel who perform technical review (must be conducted by a second qualified analyst.)*
2. Administrative review
   - *a) How to conduct a review of the report and supporting documentation to check for consistency with laboratory policies and for editorial correctness.*
   - *b) The requirements for personnel who perform administrative review (must be conducted by someone other than the analyst completing the case.)*

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Compile DNA results into a concise conclusion.

B. Write clear, concise reports that are provided to the submitting officer and prosecuting and defense attorneys.

C. Understand the purpose of case review.

D. Understand the requirements of the technical and administrative review.

E. Technically review DNA analysis cases from the testing performed to the written report.

2 Training Outline

2.1 Lesson Plan

A. Report Writing

1. General information
   - *a) Case documentation*
   - *b) Documentation, technical notes, case notes*
   - *c) Policy*
   - *d) Case-related correspondence*
   - *e) Records*
   - *f) Laboratory Reports*
   - *g) Elements in reports*
   - *h) Format of reports*
2. Submission information – Contains information about the evidence containers, date and method of submission, and relevant tracking information.
3. Requested analysis – Contains a general statement pertaining to the requested analysis, which can be a mixture of the laboratory's normally used statement and any special requests made by the submitting agency. Should reference relevant previous reports or reports previously issued in the same discipline should be included. Also, information regarding evidence transfer between laboratories or resubmissions may be included here.
4. Evidence Description, Results of Analysis and Interpretation
   - *a) Listing of items analyzed for DNA evidence.*
   - *b) Conclusions regarding characterization and interpretation of profiles.*
   - *c) Information regarding exclusion or non-exclusion of profiles from reference samples.*
   - *d) Statistical support for the conclusion.*
5. Investigative Leads - This section is directed to the investigating officer to inform them of what additional evidence needs to be submitted, such as suspect or consensual partner samples, as well as information on samples entered into CODIS.
6. Disposition – Specific disposition notes should be used to inform the officer if any further action needs to be taken on their part regarding the evidence submitted. It is also helpful to identify items that have been retained in the laboratory and those that have been depleted. This section may also be used to indicate if evidence was forwarded to another lab, or section.
7. Examination counting
8. Other types of reports
   - *a) Supplemental reports*
   - *b) Amended reports*
   - *c) Closed without analysis reports*

B. Technical Case Review

1. All case records involving the forensic examination of evidence will be technically reviewed prior to the release of results and/or issuing the case report. The supporting case information should be organized in a logical fashion with case items highlighted on relevant forms.
2. The results, interpretations, opinions, and conclusions must be reasonable, within the constraints of validated scientific knowledge, and supported by the examination records.
3. The reporting statements should be written to ensure that results are communicated properly and clearly.
4. Refer to the DNA SOP chapter: Guidelines for Technical Case Review and the Crime Laboratory Service Manual chapter: Review of Laboratory Records for the requirements of the technical review.
5. Technical review of batch records of technical data may be conducted independently of the final review of the interpretations and report. If this approach is taken by the laboratory, the technical reviewer of the interpretation and report accepts the previous review of the batch paperwork and does not perform review of this part of the case record.

C. Administrative Review

1. The administrative review will be performed on the final draft to ensure that the reported results are logical and accurate and there are no spelling or grammatical errors.
2. The chain of custody and disposition of the evidence will be reviewed for all evidence associated to the report.
3. Refer to the Crime Laboratory Service Manual chapter: Review of Laboratory Records for the requirements of the administrative review

D. Examination Counting

2.2 Required Readings

Readings are selected based on if the trainee seeks qualification in report writing, technical review, or both.

Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

A. <u>Report Writing</u>

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).
   - *a) Scope (Statement of Quality Policy);*
   - *b) Monitoring the Validity of Results;*
   - *c) Review of Laboratory Records;*
   - *d) Audits;*
   - *e) Document Management and Deviations;*

f) *Preventive Actions;*

g) *Quality Incident (QI) and Quality Action Plan Process (QAP);*

h) *Laboratory Reports, Letters, and Certificates;*

i) *Laboratory Records;*

j) *Expunction and Destruction of Laboratory Records and Information;*

k) *Electronic Storage and Archival of Records;*

l) *Records Requests and Release of Laboratory Records and Information;*

2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

   a) *Approved Standard Abbreviations List;*

   b) *Response to Quality Issues;*

   c) *Guidelines for Technical Case Review;*

   d) *Case Documentation;*

   e) *Report Writing Guidelines;*

   f) *Guidelines for Counting Examinations.*

3. Federal Bureau of Investigation. Quality Assurance Standards for Forensic DNA Testing Laboratories (most recent version). Standard 11.

4. Butler, John M. 2015. Advanced Topics in Forensic DNA Typing: Interpretation. Elsevier, Inc. Ch.16.

5. LIMS Manual. Texas Department of Public Safety Crime Laboratory (most recent version). DNA Workflow

B. Technical Review

1. Crime Laboratory Service Manual. Texas Department of Public Safety Crime Laboratory (most recent version).

   a) *Review of Laboratory Records;*

   b) *Document Management and Deviations;*

   c) *Laboratory Records;*

   d) *Laboratory Reports, Letters, and Certificates;*

   e) *Evidence and Database Sample Integrity.*

2. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).

   a) *Analytical Controls;*

   b) *Guidelines for DNA Technical Review;*

   c) *Case Documentation;*

   d) *Report Writing Guidelines.*

3. Federal Bureau of Investigation. Quality Assurance Standards Audit for Forensic DNA Testing Laboratories (most recent version). Standard 12.

3 Practice

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

3.1 Safety

None

3.2 Standards, Controls, and Reagent Preparation

None

3.3 Equipment

None

3.4 Observed Performance

A. For report writing: the trainer will provide 10-20 cases involving DNA analysis to the trainee to allow familiarity with the method of report writing in the laboratory.

B. For technical review: the trainer will demonstrate the technical review of at least one DNA analysis case.

C. For batch review of technical records: the trainer will demonstrate the batch review of at least one DNA batch consisting of technical records from multiple cases.

3.5 Independent Exercises

A. To be considered qualified in report writing, the trainee must complete these exercises, which also serves as the competency test in report writing:

1. The trainee will be provided with data (STRmix report, if applicable, and case report removed) from 20 cases previously analyzed and reported using the current validated autosomal STR amplification kit.

   a) The trainee shall use the DNA SOP and instruction from the trainer to interpret the results and, as applicable, determine what propositions will be run in STRmix. If propositions are determined, the trainee will be provided with the actual STRmix reports that were run for the case.

   b) The trainee will then prepare a case report and CODIS entry form for each case according to the format in their laboratory. Upon completion, the trainee may compare their statements with the actual statements reported on the case and discuss their conclusions/reports with the trainer.

   Note: All case information including case number, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.

2. If qualification is sought in mini-STR interpretation and reporting, the trainee will be provided with data (STRmix report (if applicable) and case report removed) from 5 cases previously analyzed and reported using the currently validated mini-STR amplification kit.

   a) *The trainee shall use the DNA SOP and instruction from the trainer to interpret the results and, if applicable, determine what propositions will be run in STRmix. If propositions are determined, the trainee will be provided with the actual STRmix reports that were run for the case.*

   b) *The trainee will then prepare a report and CODIS entry form for each case according to the format in their laboratory. Upon completion, the trainee may compare their statements with the actual statements reported on the case and discuss their conclusions/reports with the trainer.*

   ***Note*: All case information including case number, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.**

3. If qualification is sought in Y-STR interpretation and reporting the trainee must interpret the results and prepare a report for 5 sets of data developed using each of the currently validated Y-STR amplification kits for which qualification is sought.

   a) *The trainee shall use the DNA SOP and instruction from the trainer to interpret the results and prepare a report and CODIS entry form for each case according to the format in their laboratory.*

   b) *Upon completion, the trainee may compare their statements with the actual statements reported on the case and discuss their conclusions/reports with the trainer.*

   ***Note*: All case information including case number, names, and any other identifying information must be removed from the data. Case numbers and other identifying information are not included in the training record.**

4. The data sets provided as part of the 20 cases required for the *CODIS* module may be used to fulfill these requirements.

B. To be considered qualified in technical review, the trainee must complete these exercises, which also serves as the competency test in technical review:

1. If qualification is sought for technical review of autosomal STR data, the trainer will provide a minimum of 5 DNA analysis cases containing data from the current validated autosomal amplification kit on which the trainee will perform a preliminary technical review, prior to the actual case review by a qualified analyst. The set of cases should include at least one case with a mixture profile, at least one case that requires statistical significance estimation, and at least one case that requires a CODIS entry. These reviews shall be evaluated by the trainer, and the trainer will discuss any discrepancies found during the actual case review with the trainee.

   ***Note*: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.**

2. If qualification is sought for technical review of mini-STR data, the trainer will provide a minimum of 5 DNA cases containing data from the current validated mini-STR amplification kit in which the trainee will perform a preliminary technical review, prior to the actual case review by a qualified analyst. The set of cases should include at least a mixture profile, a statistical interpretation and a CODIS entry. The review will be evaluated by the trainer (mock cases may be prepared to satisfy this requirement).

   ***Note**: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.*

3. If qualification is sought for technical review of Y-STR data, the trainer will provide a minimum of 5 DNA cases containing data from each of the current validated Y-STR amplification kits in which qualification is sought. The trainee will perform a preliminary technical review, prior to the actual case review by a qualified analyst. The set of cases should include at least a mixture profile, a statistical interpretation and a CODIS entry. The review will be evaluated by the trainer (mock cases may be prepared to satisfy this requirement).

   ***Note**: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.*

4. After an evaluation of the trainee's preliminary case reviews, the trainer and/or technical lead may assign further preliminary case reviews until several cases have been reviewed by the trainee without significant discrepancies between the actual case review.

C. To be considered qualified in batch review of technical records, the trainee must complete these exercises, which also servea as the competency test in batch review of technical records:

1. If qualification is sought for batch review of technical records, the trainer will provide at least 1 DNA batch for each kit on which the trainee seeks qualification. The trainee will perform a preliminary batch review, prior to the actual batch review by a qualified analyst. This review shall be evaluated by the trainer, and the trainer will discuss any discrepancies found during the actual batch review with the trainee.

   ***Note**: All case information including case number, names, and any other identifying information is not included in the training record. Instead, a brief summary of the types of cases reviewed and trainee's performance is included.*

2. After an evaluation of the trainee's preliminary batch review of technical records, the trainers and/or Technical Leader may assign further preliminary batch reviews until several batches have been reviewed by the trainee without significant discrepancies between the actual batch review.

4 Assessment

4.1 Competency and Qualifying Examination

A. The examination will consist of the independent exercises described above. This will serve as the competency test in report writing, technical review, and batch review of technical records for DNA analysis.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer and is a prerequisite for independent report writing and technical review for DNA analysis.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

DNA-TM-06-07 LEGACY AMPLIFICATION KIT DATA: INTERPRETATION AND REVIEW

Purpose Familiarize trainees with legacy amplification kits and how updated interpretation guidelines can be used to re-interpret data (including moderate stringency CODIS hits) from these kits. Trainees may train in a single legacy amplification kit or multiple legacy amplification kits using this module. It is expected that Local CODIS Administrators that evaluate moderate stringency matches will train in all the legacy amplification kits previously used by their laboratory so they will be able to properly evaluate these types of matches.

Prerequisite Introductory Forensic Biology/DNA Unit, Theory of Analytical DNA module (unless waived), Data Analysis and Sample Characterization module, Manual Mixture Interpretation, DNA Comparison, and Match Statistics module, STRmix Mixture Deconvolution Software and Likelihood Ratios module, CODIS module, and DNA Report Writing and Case Review module.

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. The challenges and limitations involved when working with legacy amplification kit data

B. Mixture interpretation and interpretation of typing results from low quantity samples amplified with legacy kits

C. Circumstances when reinterpretation is performed and when reinterpretation will not be performed.

Note: If an analyst was previously qualified in the legacy kit (including use of dual thresholds, manual mixture deconvolution, and STRmix (if applicable)), this training is not required. The training may be modified to include only those kits in which the analyst was not previously qualified to use. If an analyst was qualified in the legacy amplification kit but did not use dual thresholds, manual mixture deconvolution, and STRmix (if applicable) during the analysis of data from the kit, then they must complete the training in order to be considered a qualified analyst in interpretation and review of legacy amplification kit data.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Analyze and interpret legacy amplification kit data using current analysis software.

B. Examine electropherograms to become familiar with recognizing the differences between true alleles and artifacts such as stutter, minus A, spikes, and pull up within the difference legacy kits.

C. Properly apply the appropriate validated mixture deconvolution protocols to legacy amplification kit data.

D. Be able to apply the skills of mixture component resolution, using a known contributor to deduce a foreign contributor, and selection of loci suitable for inclusion statistics to legacy amplification kit data.

1.3 Considerations

A. In the past, the Texas DPS Crime lab has used amplification kits including Profiler Plus, Cofiler, Identifiler, and Identifiler Plus to produce DNA profiles that were subsequently entered into the CODIS database.

B. Occasionally, these profiles result in moderate stringency hits in the CODIS database and the data must be evaluated in order to determine if the match is legitimate or simply due to the lowered stringency level.

C. Additionally, interpretation guidelines have evolved and improved, data, which would allow the possibility of utilizing more data with a reliable, validated method.

D. In some cases, not enough extract remains to amplify using new amplification kits and in other cases, it may not be necessary to amplify using new amplification kits; therefore, the original data must be used to fulfill approved requests for reinterpretation using updated guidelines.

E. In order to properly evaluate moderate stringency CODIS hits and/or reinterpret legacy amplification kit data using updated interpretation guidelines, the analysts participating in these types of evaluation must be properly trained in regards to data interpretation from legacy amplification kits.

2 Training Outline

2.1 Lesson Plan

A. Requests for reinterpretation

1. Cases containing evidentiary data run with a legacy amplification kit needing comparison to a known sample that was run with the current amplification kit
2. Cases with evidentiary data run with the current amplification kit needing comparison to a known sample that was run using a legacy amplification kit
3. CPI evaluation due to request (LIMS Manual Chapter: CPI Evaluation Reports)

B. Legacy kit loci

1. Order of loci
2. Color of loci
3. Overlapping loci between kits
4. Stutter percentages for individual loci for relevant legacy kits

C. Instrumentation used to collect and analyze legacy amplification kit data

1. 310
   - *a) Matrix files*
   - *b) How to create and apply a new matrix file to data*
   - *c) Mac data versus PC data*
2. 3100/3130
3. Genescan/Genotyper
   - *a) Analysis range*

b) *Size standards*

c) *Panels*

4. GeneMapper

a) *Analysis range*

b) *Size standards*

c) *Panels*

5. Second review of raw data by a qualified analyst

6. Positive control profiles for each legacy kit

D. Analytical and Stochastic thresholds for each legacy kit

E. Use of statistics by the Texas DPS Crime Lab

1. RMP tool for single source significance calculation (RMP and 2p-p2)

2. Popstats for mixed source significance calculation (CPI)

2.2 Required Readings

A. Readings are selected based on the amplification kits for which the trainee seeks qualification. The readings in this section are required for all trainees.

B. Readings that have been completed in previous modules may be omitted or reviewed at trainer's discretion.

1. SWGDAM Clarification on the Reinterpretation of Data Typed with Legacy Amplification Test kits (SWGDAM – June 6, 2016)

2. LIMS Manual. Texas Department of Public Safety Crime Laboratory (most recent version). CPI Evaluation Reports

3. Budowle, et al. Source Attribution of a Forensic DNA Profile. For Sci Comm. 2000 2(3).

4. Any local policies as applicable.

2.3 Conditional Readings

These readings are required if the trainee seeks qualification for the amplification kit described in the reading.

A. <u>Profiler Plus/Cofiler</u>

1. Profiler Plus User's Manual (Vendor – 2012)

2. Cofiler User Bulletin (Vendor – 2012)

3. Instrument Validation/Profiler Plus Validation (System – 1998)

4. Cofiler Validation (System – 1998)

5. Any local implementation validation studies – Profiler Plus, Cofiler

6. Profiler Plus and Cofiler Supplemental Validation Study (Valid-Method-SYS-DNA-ProfilerPlusCofiler- 2017-0703 and Valid-Method-SYS-DNA-ProfilerCofiler-2017-1030)

a) *Valid-Method-SYS-DNA-AppliedBiosystems-ProfilerPlusCofiler-2017-1030-1of3*

b) *Valid-Method-SYS-DNA-AppliedBiosystems-ProfilerPlusCofiler-2017-1030-2of3*

c) *Valid-Method-SYS-DNA-AppliedBiosystems-ProfilerPlusCofiler-2017-1030-3of3*

7. Archived DNA SOP (Profiler Plus/Cofiler) 2003-1110-2004-0309 (pages 138-152; 224-235)

B. Identifiler

1. Identifiler User Guide (Vendor – 2012)
2. Local validation study - Identifiler
3. Identifiler Supplemental Validation Study (Valid-Method-SYS-DNA-Identifiler-2017-1110)
4. Archived DNA SOP (Identifiler) 2008-0415-2008-1201 (pages 147-149)

C. Identifiler Plus

1. Identifiler Plus User Guide (Vendor – 2015)
2. Identifiler Plus System Validation Study (Valid-Method-LUB-DNA-IdentifilerPlus-2011-0321)
3. Dual Thresholds Validation Study (Valid-Method-SYS-DNA-DualThresholds-Summary-2015-0803)
4. Any local implementation validation studies – Identifiler Plus
5. Archived DNA SOP (Identifiler Plus) 2017-1024-2017-1201 (pages 243-245)
6. Mixture Validation Study (Valid-Software-SYS-DNA-DPSMixtureDeconvolutionTool-2016-0212-1of2 and-2of 2

3 Practice

3.1 Supervised Exercises

The trainee will attend a lecture for each legacy amplification kit for which they intend to qualify provided by an analyst previously qualified in interpretation of legacy amplification kit data OR a lecture provided by a Technical Leader who was trained by an analyst previously qualified in interpretation of legacy amplification kit data.

4 Assessment

4.1 Competency and Qualifying Examination

The trainee will be given at least 5 sets of DNA data run with Profiler Plus/Cofiler, at least 5 sets of DNA data run with Identifiler, and at least 5 sets of DNA data run with Identifiler Plus.

Competency sets may be selected based on which kit the analyst was not previously qualified to use and on which kit the trainee intends to qualify.

A. Based on their understanding of the DNA SOP STR Interpretation Guidelines, the trainee should

1. assign a number of contributors,
2. characterize the profile as full or partial,
3. determine if a major component is present,
4. calculate the average mixture ratio,

5. indicate loci suitable for statistics,
6. determine which individuals would be excluded or included for each set of data, and
7. describe which statistic(s) would be used to determine the significance of the association, as applicable.

B. Identifiler Plus data may be analyzed using STRmix software.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Interpretation Unit Training Checklist (LAB-DNA-TM-05).

07 DNA SUPPORT STAFF TECHNICIAN UNIT

DNA-TM-07-01 REAGENT PREPARATION AND QUALITY CONTROL FOR TECHNICIANS

Duration 3 to 14 days

Purpose To provide training in how to make reagents and perform quality control checks on reagents used in the biology/DNA section of the laboratory.

Prerequisite Successful completion of the Introductory Forensic Biology/DNA Unit

1 Objectives

1.1 Theoretical

Upon completion of this training, the trainee will have knowledge of:

A. How proper preparation and documentation of reagent preparation is crucial to demonstrating quality control in the laboratory.

B. How quality control checks are essential to demonstrating proper preparation of reagents prior to their use on casework samples.

C. The potential impact of not performing quality control checks on reagents.

1.2 Practical

Upon completion of this training, the trainee will be able to:

A. Prepare reagents commonly used in the Biology and DNA sections of the laboratory.

B. Prepare and complete proper documentation for prepared reagents.

C. Perform quality control checks on purchased and prepared reagents as necessary.

D. Determine which reagents require quality control checks.

E. Prepare and complete proper documentation for quality control checks on reagents.

F. Determine what actions to take in order to release a reagent for use on casework.

2 Training Outline

2.1 Lesson Plan

A. Blood tests

1. Types of tests used by the laboratory

   a) TMB

   b) PHT

   c) LMG

   d) Luminol

   e) Hematrace

2. Proper storage conditions for reagents and components of reagents; determination of expiration dates

3. Quality testing
   a) *Use of proper controls*
   b) *Procedure*
   c) *Pass/fail criteria*
   d) *Documentation*
4. Proper documentation of reagent preparation

B. Semen tests
1. Types of tests used by the laboratory
   a) *Acid phosphatase*
   b) *Nuclear Fast Red*
   c) *Picroindigocarmine*
   d) *ABAcard p30*
2. Proper storage conditions for reagents and components of reagents; determination of expiration dates
3. Quality testing
   a) *Use of proper controls*
   b) *Procedure*
   c) *Pass/fail criteria*
   d) *Documentation*
4. Proper documentation of reagent preparation

C. DNA extraction reagents
1. DNA reagents that may be prepared in the laboratory
   a) *Chelex 5% and/or 20%*
   b) *Digest buffer*
   c) *DTT 0.39M and/or 1M*
   d) *Proteinase K solution*
   e) *20% Sarcosyl solution*
   f) *5N Sodium Hydroxide*
   g) *Stain Extraction Buffer*
   h) *TE^{-4} buffer*
   i) *TNE Solution*
2. Purchased DNA reagents that may require subsequent laboratory preparation prior to use
   a) *PrepFiler Wash Buffer A and B*
   b) *QIAamp Buffer AW1 and AW2*

3. Proper storage conditions for reagents and components of reagents; determination of expiration dates
4. Quality testing
   a) *Use of proper controls*
   b) *Procedure*
   c) *Pass/fail criteria*
   d) *Documentation*
5. Proper documentation of reagent preparation

D. Notification of Technical Leader to determine if pass criteria is met and to release solution for use.

2.2 Required Readings

Readings are selected based on the solutions for which the trainee seeks authorization to prepare and quality control check. The readings in this section are required for all trainees.

A. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version).
   1. Facilities;
   2. Analytical Controls;
   3. Critical Reagents;
   4. Commercial Reagents.

B. Crime Laboratory Service Manual Laboratory Equipment chapter

2.3 Conditional Readings

These readings are required if the trainee seeks authorization to prepare and quality control check the solution described in the reading.

A. <u>TMB</u>
   1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Blood Tests – PHT, TMB, and LMG, Tetramethylbenzidine Solution, Tetramethylbenzidine (TMB) Reagent Preparation Form.
   2. Safety Data Sheet for 3,3,5,5-Tetramethylbenzidine

B. <u>Luminol</u>
   1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Blood Test - Luminol, Luminol Reagent Preparation Form.
   2. Safety Data Sheet for Luminol

C. <u>Hematrace</u>
   1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Species Origin Test – Hematrace, ABAcard Reagent Quality Control.

2. Abacus Diagnostics. ABAcard HemaTrace For the Forensic Identification of Human Blood. Technical Information Sheet. Abacus Diagnostics (most recent version).

D. Leucomalachite Green

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Blood Tests – PHT, TMB, and LMG, Leucomalachite Green Solution, Leucomalachite Green (LMG) Reagent Preparation Form.

2. Safety Data Sheet for Leucomalachite Green

E. Phenolphthalin

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Blood Tests – PHT, TMB, and LMG, Phenolphthalin Solution, Phenolphthalin (PHT) Reagent Preparation Form.

2. Safety Data Sheet for Phenolphthalin

F. Acid Phosphatase

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Presumptive Semen Test – Acid Phosphatase, AP Test Reagent, Acid Phosphatase Reagent Preparation Form.

2. National Institute for Occupational Safety and Health (NIOSH). Health hazard alert – benzidine – 0-tolidine-, and o-dianisidine- based dyes. Department of Health and Human Services Publication number 81-106. 1980. pp. 1-7.

3. Safety Data Sheets for α-naphthyl acid phosphate, calcium salt, glacial acetic acid, Aerosol 22, o-dianisidine, tetrazotized, sodium chloride

G. Nuclear Fast Red

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Body Fluid Stain Extraction, Spermatozoa Examination, Nuclear Fast Red Solution, Nuclear Fast Red Reagent Preparation Form.

2. Safety Data Sheets for aluminum sulfate and nuclear fast red

H. Picroindigocarmine

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Body Fluid Stain Extraction, Spermatozoa Examination, Picroindigocarmine, PIC Reagent Preparation Form.

2. Safety Data Sheets for indigocarmine and picric acid or saturated picric acid solution

I. ABAcard p30

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Body Fluid Stain Extraction, P30 Identification, ABAcard Reagent Quality Control.

2. Abacus Diagnostics. ABAcard p30 Test for the Forensic Identification of Semen. Technical Information Sheet. Abacus Diagnostics. (most recent version)

J. Chelex 5% and 20%

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). DNA Extraction with Chelex, Chelex 5% and 20%, Chelex 5% and 20% Reagent Preparation Form.
2. Safety Data Sheet for Chelex

K. Digest Buffer

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). DNA Extraction with Digest Buffer – Organic, Differential Extraction with Digest Buffer, Digest Buffer, Digest Buffer Reagent Preparation Form.
2. Safety Data Sheet for 1M Tris-HCl, 0.5 M EDTA, and 20% Sodium dodecyl sulfate

L. DTT – 0.39M/1M

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). DTT – 0.39M/1M, DTT – 0.39M/1 M Reagent Preparation Form.
2. Safety Data Sheet for DTT

M. Proteinase K Solution

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Proteinase K Solution, Proteinase K Reagent Preparation Form.
2. Safety Data Sheet for Proteinase K

N. 20% Sarcosyl Solution

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Sarcosyl Solution – 20%, Sarcosyl Solution – 20% Reagent Preparation Form.
2. Safety Data Sheet for Sarcosyl

O. Sodium Chloride – 5M

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Sodium Chloride – 5M, 5M NaCl Reagent Preparation and Quality Control.
2. Safety Data Sheet for Sodium Chloride

P. Sodium Hydroxide – 5N

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Sodium Hydroxide – 5N, 5N NaOH Reagent Preparation and Quality Control.
2. Safety Data Sheet for Sodium Hydroxide

Q. Stain Extraction Buffer

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). DNA Extraction with Stain Extraction Buffer –

Organic, Stain Extraction Buffer, Stain Extraction Buffer (SEB) Reagent Preparation Form.

2. Safety Data Sheet for Tris base, 0.5M EDTA solution, sodium dodecyl sulfate, concentrated hydrochloric acid, DTT

R. TE-4 Buffer

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). TE-4 Buffer, TE-4 Buffer Reagent Preparation Form.

2. Safety Data Sheet for 1M Tris-HCl, 0.5M EDTA solution

S. TNE Solution

1. DNA Standard Operating Procedures. Texas Department of Public Safety Crime Laboratory (most recent version). Differential Extraction with TNE, TNE Solution, TNE Reagent Preparation Form.

2. Safety Data Sheet for 1M Tris-HCl, 5M NaCl, 0.5M EDTA

3 Practice

3.1 Safety

A. Gloves must be worn during reagent preparation and testing. Clothing may protect unbroken skin; broken skin should be covered. Eye protection is recommended during reagent preparation and handling of liquid body fluids. Chemicals used may be carcinogenic or caustic. Blood may contain infective agents. Use universal precautions during evidence handling.

B. Zinc, used in the preparation of leucomalachite green and phenophthalin solutions, may burn on exposure to oxygen. Follow preparation instructions and warnings carefully. Always wear appropriate eye protection while using the alternate light source and avoid looking directly into the light. Follow safety and use directions provided with the instrument.

C. Picric acid, used in the preparation of picroindigocarmine stain, is explosive when dry. Follow storage and preparation instructions and warnings carefully.

D. α-naphthyl acid phosphate and o-dianisidine are suspected carcinogens. Application of the AP spot test reagent using a spray bottle must be performed in a chemical fume hood.

3.2 Standards, Controls, and Reagent Preparation

A. Standards and Controls

1. For blood and semen test reagents

a) On the day of use, test blood presumptive reagents/acid phosphatase using a positive and negative control. Record results on the Biological Screening Worksheet (LAB-DNA-01) or LIMS equivalent.

b) Prior to first use, each new lot of ABAcard Hematrace test kits/ABAcard p30 test kits must be tested according to HemaTrace Reagent Quality Control form (LAB-DNA-09).

c) Prior to first use, each new lot of NFR/PIC must be tested according to NFR Solution Reagent Preparation and Quality Control (LAB-DNA-29) and PIC

Solution Reagent Preparation Quality Control (LAB-DNA-42). This does not apply to purchased reagents.

d) *The lot number and expiration date of all reagents used for testing must be documented in the case record.*

2. For DNA extraction reagents

a) *Documentation and use of required forms.*

b) *Reagent blanks for each set of extraction as noted in the relevant DNA SOP chapter.*

c) *Extraction kits as critical reagents.*

B. Reagent Preparation

1. Blood test reagents as applicable: TMB, PHT, LMG, Luminol
2. Semen test reagents as applicable: AP, NFR, PIC
3. DNA extraction reagents as applicable: stain extraction buffer, digest buffer, Chelex, TNE, Sarcosyl, Proteinase K, 0.39 M and 1M DTT, TE-4 buffer, 5M NaCl, 5N NaOH
4. Subsequent preparation of DNA extraction reagents as applicable: PrepFiler Wash Buffer A and B, QIAamp Buffer AW1 and AW2.

3.3 Equipment

- Glassware
- Spatulas/scoops
- Balance
- Hot Plate
- Stir Plate
- Magnetic Stir Bar
- Litmus paper
- Pipettes
- Oven
- Vortex

4.3 Observed Performance

A. Trainer will discuss the procedures for preparation of relevant solutions including correct documentation and labeling.

B. Trainer will discuss how to perform quality control procedures on relevant reagents including appropriate documentation of quality control checks.

C. Trainer will discuss how to perform quality control procedures on ABAcard Hematrace and ABAcard p30 tests as applicable to their laboratory including appropriate documentation of quality control checks.

3.4 Supervised Performance

A. The trainee will prepare relevant solutions under the direct supervision of the trainer.

1. Trainee will document reagent preparation on the relevant reagent preparation log.
2. Trainee will perform proper labeling on container for each solution prepared.

B. The trainee will perform quality control procedures on the relevant reagents that they prepared based on laboratory policy under the direct supervision of the trainer. Trainee will document reagent quality control on the appropriate forms.

C. The trainee will perform the quality control procedure for ABAcard Hematrace and ABAcard p30 tests as applicable to their laboratory under the direct supervision of the trainer. Trainee will document the quality control on the appropriate forms.

3.5 Independent Exercises

Trainee will independently perform the following tasks to demonstrate competency in reagent preparation:

A. Use a balance to weigh 0.1 g, 1 g, and 10 g of a substance.

B. Use a pipette to distribute the following amounts of a liquid into individual microcentrifuge tubes: 5µL, 15µL, 50µL, and 900µL.

C. Use the appropriate lab equipment to measure 86 mL, 178 mL, and 990 mL of liquid.

4 Assessment

4.1 Competency and Qualifying Examination

A. The independent exercise above is the competency test in reagent preparation.

B. The trainer will administer a written examination. Incorrect responses will be reviewed and/or remediated with the trainee. Successful completion of this module is determined by the trainer.

4.2 Evaluation of Training

The trainee and trainer will complete the DNA Technician Training Checklist (LAB-TM-DNA-06).

08 FORMS

TRAINING FORMS

	Document Name	FRN
1	Introductory Forensic Biology/DNA Unit Training Checklist	LAB-DNA-TM-01
2	Biological Evidence Screening Unit Training Checklist	LAB-DNA-TM-02
3	Male Screening Unit Training Checklist	LAB-DNA-TM-03
4	Analytical DNA Unit Training Checklist	LAB-DNA-TM-04
5	DNA Interpretation Unit Training Checklist	LAB-DNA-TM-05
6	DNA Technician Training Checklist	LAB-DNA-TM-06
7	Approval Checklist: Analysts/Technicians with Previous Forensic Experience	LAB-DNA-TM-07