

**AUSTIN POLICE DEPARTMENT
SEROLOGY/ DNA SECTION
TRAINING MANUAL**

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Overview of training program (QAS 5.1.2)

Examiners will be provided fundamental serology (screening and body fluid identification) and DNA analysis training (when applicable), which will include protocols in the SOP and Technical Manual that are used in the lab. Serology and DNA training will be undertaken as separate units of training and will each conclude when:

- All competency samples are correctly analyzed;
- The competency notebook is approved by the Technical Leader;
- The competency notebook, other training records documenting completion of training requirements, and trainee credentials are approved by the Division manager;
- The trainee successfully completes a written exam administered by the Technical Leader or trainer.
- The laboratory supervisor and trainer(s), when applicable, recommend that the examiner be approved for independent casework;
- The Technical Leader and Quality Assurance manager recommends that the examiner be approved for independent casework; and
- The Laboratory Director approves the examiner for independent casework.

Prior to completing DNA analysis training, examiners must additionally complete:

- Fifty (50) screening and/or body fluid identification cases.
- Six months of Forensic DNA analysis experience;
- One to five previously examined, adjudicated cases or mock cases, at least one of which includes a differential extraction of semen evidence, and the results of which must essentially agree with those of the qualified examiner; and
- The trainee will review a minimum of 10 sets of data representative of casework and provide a written interpretation of the data according to the laboratory policy.
- Moot court to be evaluated by the trainer or the trainer's appointee.

At the discretion of the laboratory supervisor, a trainee may conduct casework analyses on evidence, *except* for the interpretation of results, formation of conclusions, generation or review of reports, or court testimony requiring interpretation of results or formation of conclusions. Competence in any analysis *must* be demonstrated by practical exam and documented prior to unsupervised performance.

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Analysis training will be conducted according to the Training Manual. The Technical Leader has the authority to determine if some steps or processes in the Training Manual are not required for that particular trainee. In such cases, a memo will be included indicating the reason for the adjustments. Training beyond what is required in the training manual will be performed at the discretion of the Technical Leader and Supervisor. The Technical Leader is responsible for proposing and reviewing changes to the Training Manual. The DNA Technical Leader is responsible for maintaining the Training Manual, evaluating proposed changes and presenting those proposals for approval as appropriate. Experienced analysts new to the lab, at the discretion of the technical leader, may bypass the formal training program and immediately begin the competency test.

COMPETENCY TESTING

The trainee demonstrates competence in examinations through the preparation and presentation of a competency notebook of properly analyzed unknown (to the trainee) samples and the successful completion of a written examination. Previously approved analysts do not need to take the written test and can solely perform the practical test.

- **Competency notebook**

The DNA Technical Leader is responsible for creating, maintaining, and distributing training and competency samples as well as records of their sources and known types.

The trainee will examine the competency samples according to the SOP and Technical Manual, maintaining analysis documentation as for casework.

For serology analysis training, the competency samples will include a minimum of five stains and/or swabs for each of the training areas of blood and semen identification.

For DNA analysis training, the competency samples will include a minimum of ten bloodstains, ten buccal swabs, five mixed stains of semen and epithelial cells for differential extraction, and five hairs (optional). The trainee may request additional hairs as needed.

The Technical Leader will review the competency notebook prior to its presentation for review by the DNA Supervisor.

The competency notebook and/or training notebook will include at a minimum:

- The training log showing the start of training and the activities and date on each day of training;
- Analysis documentation for all competency samples; and

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- Written tests taken during training.

Other training records, such as practice sample analysis documentation, may be kept in the competency notebook.

- **Written examination**

The trainee must successfully complete a written examination covering the entire training unit of either serology or DNA prior to approval to perform independent analyses. The exam will be designed to document the trainee's knowledge of the procedures as well as the trainee's understanding of the theoretical basis for those procedures. In addition, the trainee may be asked to demonstrate knowledge and understanding of quality assurance practices relevant to his or her work. The exam will be administered by the DNA Technical Leader or trainer.

- **Unusual samples**

An approved examiner may use a valid procedure for analysis of a body fluid, tissue, bone or teeth not encountered during training providing the analyst has previously demonstrated competence in that procedure.

- **Experienced examiner competency testing of approved procedures**

Successful completion of a competency test is required prior to casework processing when an experienced DNA analyst begins employment at the APD DNA lab. An experienced examiner is required to test 5 previously analyzed samples in each method in order to demonstrate competency. Documentation will be retained in the laboratory.

- **Experienced examiner competency testing for new procedures**

Each experienced examiner will perform a minimum of 5 competency samples prior to using the new method in casework.

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MODULE 1 GENERAL LABORATORY PRACTICES

DURATION 1 to 2 days

PURPOSE Provide an orientation of the practices in the Austin Police Department
Forensic Science Division

1.1 OBJECTIVES

1.1.1 THEORETICAL

Trainee will become familiar with the overall operations of the Austin Police Department Forensic Science Division.

Trainee will become familiar with the APD Policy Manual, the Forensic Science Division SOP, and Safety Manual.

1.1.2 PRACTICAL

Trainee will be able to:

- Discuss the organization and chain of command in APD and in the Forensic Science Division.
- Discuss the objective and mission of APD.
- Discuss the objective and mission of the Forensic Science Division..

1.2 OUTLINE

() refer to associated references

- I. Overview of APD operations (1)
 - A. Introduction to the APD Policy Manual
 - B. Organization and administration
 - C. Objective, mission and program
 - D. Professional conduct and ethics
- II. Overview of Crime Laboratory Operations (2)
 - A. Standard Operating Procedures
 - B. Employee Development Program
 1. Training Program
 2. Employee Career Development

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- III. General office practices and basic office equipment
 - A. Telephones
 - B. Work hours, lunch hours, breaks
 - C. Computers – security and operation
- IV. Identification
 - A. APD I.D.
- V. Building security (2)
 - A. Building security
- VI. Laboratory security (2)
- VII. Travel
- VIII. Release of information and open records act (2)
- IX. Ethics (3), (4)

1.3 THEORY

As a new employee, it is important to become acquainted with the written directives, orders, and other information necessary to insure effective operation within the Forensic Science Division as well as the Austin Police Department as a whole. The APD Policy Manual and Forensic Science Division SOP will provide the new employee with valuable information and instruction on which to build his/her career and how to conduct themselves daily.

1.3.1 READINGS

- (1) Austin Police Department Policy Manual
- (2) Forensic Science Division SOP
- (3) To Hell and Back: The Ethics of Stewardship and the Stewardship of Ethics
- (4) ASCLD/LAB “Guiding Principles of Professional Responsibility for Crime Laboratories and Forensic Scientists”

1.4 PRACTICE

None

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1.5 WRITTEN TEST

None

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MODULE 2 INTRODUCTION TO FORENSIC SCIENCE

Duration 1/2 to 1 day

Purpose Provide an orientation and overview of the discipline of forensic science.

2.1 OBJECTIVES

2.1.1 THEORETICAL

The trainee will be able to:

- Define forensic science.
- Discuss the roles of the forensic scientist.

Discuss from a historical point of view the individuals and their specific contributions to formulating the disciplines that now constitute forensic science.

- Outline the major disciplines in forensic science.
- Define physical evidence and list common types of physical evidence
- Explain the concepts of identification and individualization.
- Discuss the criminal justice system and the role of the expert witness within it.
- Discuss quality assurance as it applies to the forensic laboratory.

Example assessment question: Explain Locard's Exchange Principle.

2.1.2 PRACTICAL

None

2.2 OUTLINE

() refer to associated references

- I. Science and the law **(1, pp. 1-35), (2, pg. 3)**
 - A. History and development
 - B. Physical evidence, circumstantial evidence **(1, pp. 66-96)**
 - C. Scientific method

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- D. Reconstruction through associations
- II. Identification and individualization **(1, pp. 69-76), (2, pp. 3-5)**
 - A. Class characteristics
 - B. Individualizing characteristics
- III. Transfer theory **(2, pg. 5)**
 - A. Comparison and association
 - B. Elimination and inclusion
- IV. Disciplines **(1, pp. 11-14)**
 - A. Latent prints
 - B. Questioned documents
 - C. Drugs
 - D. Toxicology
 - E. Trace evidence
 - F. Forensic biology
 - 1. Serology
 - 2. DNA
 - 3. Entomology
 - G. Firearms/Toolmarks
 - H. Multi-media
 - I. Crime Scene Investigations
 - 1. Crime Scene Speciality
 - 2. Property Crimes
 - J. Polygraph
- V. Criminal law enforcement **(1, pg. 8-10), (3)**
 - A. Agencies and jurisdictions
 - B. Courts
 - C. Criminal trial process
- VI. Trends
 - A. QA **(2, pp. 139-144)**
 - B. Testimony admissibility **(2, pp. 145-155)**

2.3 THEORY

An understanding of the historical development of the field of forensic science is fundamental to the crime laboratory analyst. The forensic scientist must understand the application of science to the laws that govern our society and that are enforced through our criminal justice system.

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2.3.1 READINGS

- (1) Saferstein, Richard. 2001. *Criminalistics: An Introduction to Forensic Science*, 6th ed. Prentice Hall, Inc. Pp. 1-35, 66-96.
- (2) Inman, Keith and Norah Rudin. 1997. *An Introduction to Forensic DNA Analysis*. CRC Press LLC. Pp. 3-5, 139-155.
- (3) Kuzmack, Nicholas T. 1982. *Legal Aspects of Forensic Science*. *In: Forensic Science Handbook*. Richard Saferstein, ed. Prentice-Hall, Inc. Chapter 1, pp 1-18.

2.4 PRACTICE

None

2.5 WRITTEN TEST

None

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MODULE 3 INTRODUCTION TO SEROLOGY / HISTORY OF SEROLOGY

Duration 1 to 2 days

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

3.1 OBJECTIVES

3.1.1 THEORETICAL

Trainee will be able to:

- Define and understand basic genetic, biological, and immunological terms.
- Discuss from a historical point of view the major developments in forensic biology.
- Discuss advances in forensic serology.

3.1.2 PRACTICAL

None

3.2 OUTLINE

() refer to associated references

- I. Introduction to forensic serology (**1, pages 20-65**)
 - A. Definition, importance, legal value
 - B. Basic biochemistry (amino acids, proteins, enzymes)
 - C. Basic genetics (mitosis, meiosis, mutations, polymorphisms)
 - D. Basic immunology (antigen/antibody concepts)
- II. History of forensic serology (**3, pages 361-436**)
 - A. Heredity
 - B. Early blood and semen discoveries (**1, pages 257-274**)
 - C. Genetic markers of the red cell (**4**)
 1. Red cell antigens
 - a) Techniques for testing
 - b) Issues of concern, limitations

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- 2. Red cell isoenzymes and proteins **(1, page 637)**
 - a) Techniques for testing
 - b) Issues of concern, limitations
- D. Genetic markers of the plasma
 - 1. Plasma ABO antibodies and antigens
 - a) Techniques for testing
 - b) Issues of concern, limitations
 - 2. Plasma proteins
 - a) Techniques for testing
 - b) Issues of concern, limitations
- E. Genetic markers of semen and saliva **(5)**
 - 1. Soluble antigens **(2)**
 - a) Techniques for testing
 - b) Issues of concern, limitations
 - 2. Isoenzymes in semen (PGM)
 - a) Techniques for testing
 - b) Issues of concern, limitations
- F. Other body fluids

3.3 THEORY

The ability to understand and use proper biological terminology is necessary for successful communications of forensic serology in the courts. The history of the developments and past methods in forensic serology will provide a foundation to the concepts and processes used toward the individualization of a forensic stain.

3.3.1 READINGS

- (1) Gaensslen, R.E. 1983. Sourcebook in Forensic Serology, Immunology, and Biochemistry. Pp. 20-65, 257-274, 637.
- (2) Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence. 1983. Pp. 91-111.
- (3) Saferstein, Richard. 2001. Criminalistics: An Introduction to Forensic Science, 6th ed. Prentice Hall, Inc. Pp. 320-352.
- (4) Saferstein, Richard. 1982. Forensic Science Handbook. Pp. 267-270, 320-325, 338-346.
- (5) Saferstein, Richard. 1988. Forensic Science Handbook, Vol. II. Pp. 369-381.

3.4 PRACTICE

None.

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3.5 WRITTEN TEST

None

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Human Identity PCR Testing Chronology

1983	Kary Mullis invents PCR
1986	First PCR DNA case in U. S. criminal court (PA vs. Pestnikas) using HLA DQ α typing results
1991	D1S80 Forensic DNA Amplification Reagent Set introduced
1993	QuantiBlot™ Human DNA Quantitation Kit introduced Kary Mullis wins Nobel Prize in Chemistry
1998	DPS began casework on STRs

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MODULE 4 QUALITY ASSURANCE

Duration ½ to 1 day

Purpose Educate trainee on the principles of quality assurance as they apply to forensic Serology/DNA .

4.1 OBJECTIVES

4.1.1 THEORETICAL

Trainee will be able to:

- Define and understand the basic principles of quality assurance.
- Understand the accreditation process.
- Describe the elements involved in assuring product quality
- Describe the elements involved in demonstrating lab competence and assessing individual competence.

4.1.2 PRACTICAL

None

4.2 OUTLINE

() refer to associated references

- I. Introduction
 - A. Objectives of QA
 1. Quality System **(1)**
 2. Quality Assurance **(2)**
 - B. Responsibility of QA **(1)**
 - C. Quality System Review **(1)**
- II. Assessing individual competence
 - A. Training and Examiner Approval **(1)**
 1. Criminalist Development **(1)**
 2. Training **(2)**
 - B. Competency and proficiency testing
 1. Proficiency Testing **(1)**
 2. Competency Testing **(2)**
 3. The Role of Proficiency Testing and Audits **(3)**

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- 4. Proficiency Testing **(4; standard 13)**
- C. Peer review and testimony monitoring
 - 1. Court Testimony Monitoring **(1), (2)**
 - 2. Review of casework **(1), (2)**
- III. Demonstrating lab competence
 - A. Validation **(1), (2), (4;standard 8)**
 - B. Audits **(1), (2), (4;standard 15)**
 - C. Accreditation
 - 1. ASCLD/LAB **(2)**
 - 2. NFSTC
- IV. Insuring product quality
 - A. QC
 - 1. Ensuring High Standards of Laboratory Performance **(3)**
 - 2. Safeguarding Against Error **(3)**
 - B. Standards, controls and reagents **(1), (2)**
 - C. Technical Problems **(2)**
 - D. Technical review **(1), (4;standard 12)**
 - E. Administrative review **(1)**
- V. Scientific working groups
 - A. SWGDAM **(2)**
 - B. AFDA
- VI. DNA specific QA
 - A. Quality Assurance Program **(4;standard 3)**
 - B. DNA Advisory Boards **(2)**
 - C. NDIS **(2)**
 - D. NIST **(2)**

4.3 THEORY

The ability to understand and apply proper quality assurance practices is essential to the process of forensic analysis. Knowledge of governing bodies from local to national levels is important. The development, coordination, and maintenance of reliable, uniform and scientifically sound laboratory procedures is dependent upon a solid quality assurance program. Formal methods of quality assurance insure compliance with ASCLD/LAB accreditation standards.

4.3.1 Readings

- (1)** Forensic Science Division SOP
- (2)** Serology/DNA Unit SOP and Technical Manual

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(3) National Research Council. 1996. The Evaluation of Forensic DNA Evidence. Washington, D.C. National Academy Press. 1996. Pp. 75-78, 78-80, 80-85.

(4) Quality Assurance Standards for Forensic DNA Testing Laboratories. October 1, 1998.

Standard 3 Standard 13 Standard 12
Standard 8 Standard 15

4.4 PRACTICE

None

4.5 Written test

None

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Module 5 General Safety

Duration 2 to 3 days

Purpose Orient and acquaint the trainee with general laboratory safety practices to be followed in the laboratory.

5.1 OBJECTIVES

5.1.1 Theoretical

Trainee will be able to locate and properly use the safety equipment located in the crime laboratory (eyewash, first aid kit, emergency shower, fire blanket, fire extinguishers, etc.)

Trainee will know the location of the laboratory safety manual, chemical hygiene plan, exposure control plan and the material safety data sheets in the laboratory and be familiar with the contents of each.

Example assessment question: Where would you find the toxicity data and exposure limits for Chloroform?

5.1.2 Practical

Trainee will be able to operate safety devices in the laboratory.

Trainee will be able to refer to the appropriate MSDS when necessary

5.2 OUTLINE

() refer to associated references

- I. Intro to safety devices and accident reporting
 - A. Fire extinguishers
 1. Location in laboratory
 2. Using a Fire Extinguisher
 3. Fire fighting equipment
 - B. Eyewash/Emergency shower
 1. Location in laboratory
 2. Eyewashes and Safety Showers
 - C. Fire blankets
 1. Location in laboratory
 2. Fire Safety

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- D. Housekeeping
- E. Ventilation
- F. Laboratory Equipment Hazards
- G. Safety Equipment and Clothing
- H. General Lab Safety Tips
 - 1. Personal Hygiene
- I. Reporting accidents
 - 1. Employees Exposed to Communicable Diseases **(4)**
 - Reporting Work-Related Injuries and Infectious Disease Exposures **(4)**
 - 2. Exposure Evaluations and Medical Consultations **(4)**
 - 3. Exposure to Infectious Biohazards **(4)**
 - 4. Exposure Incident **(4)**
- J. QC documentation of safety devices
 - 1. Safety Inspections
- II. Biological hazards
 - A. Universal precautions **(1)**
 - 1. Work Related Exposure to Infectious Disease
 - 2. Protective Apparel
 - 3. Laboratory Safety Guidelines for Prevention of Disease
 - 4. Engineering and Work Practice Controls
 - 5. Personal Protective Equipment
 - B. Pathogens **(1)**
 - 1. Information on AIDS
 - C. Hepatitis vaccination, HIV testing
 - 1. Guidelines for Payment of Expenses Associated with Exposure To Infectious Disease **(4)**
 - 2. Recordkeeping
 - 3. Hepatitis B vaccination and Post Exposure Follow-up
- III. Chemical hazards
 - A. Handling
 - 1. Workplace Chemical List
 - 2. Hazard Communication Program **(1; HAZCOM Program)**
 - 3. Chemicals **(1)**
 - 4. Methods of Biological and Chemical Contamination **(1)**
 - B. Material Safety Data Sheets **(2)**
 - C. Signs and Labeling **(1)**
 - D. Storage **(1; HAZCOM Program), (1)**
 - 1. Safety Cans
 - 2. Flammable Storage Cabinets

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- 3. Corrosive Storage Cabinets
- IV. Hazardous waste
 - A. Chemical waste disposal and spill clean-up **(1; HAZCOM Program), (1)**
 - 1. Spills and Accidents
 - 2. Emergency Plans
 - 3. Disposal/spill control
 - B. Biological waste disposal and spill clean-up **(1)**
- V. Alternative light sources **(1)**
- VI. Emergency response
 - A. Internal
 - 1. Evacuation Plans for Department Occupied Facilities
 - 2. Response to Bomb Threats
 - 3. Severe Weather and Tornado Reporting
 - B. External

5.3 THEORY

Proper safety practices must always be followed when handling chemicals or biological materials. Safety responsibility begins with each individual and extends to the entire workplace. Safety requires a personal commitment and desire for self-protection.

5.3.1 Readings

- (1)** Forensic Science Division Safety Manual
- (2)** MSDS's (Manufacturer's Safety Data Sheets)
- (3)** Safety Guidelines (International Association for Identification)
- (4)** Austin Police Department Policy Manual

5.4 PRACTICE

5.4.1 Safety

Wear gloves and lab coats when working in the laboratory. Safety glasses or goggles and mask may be required during some operations. Safety equipment will be issued to the trainee. The biological safety cabinet (Laminar flow hood) should be used when handling potentially hazardous biological samples.

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5.4.2 Quality control

Laboratory safety checklist schedule

5.4.3. Basic Skills

Trainer will review the laboratory safety checklist.

5.5 Written test

A written test will be administered by the trainer.

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Module 6 BASIC EQUIPMENT USE AND QUALITY CONTROL

Duration 2 to 4 days

Purpose Familiarize trainee with general equipment available in the laboratory.

Enable student to safely operate, calibrate, clean and maintain laboratory equipment.

6.1 OBJECTIVES

6.1.1 Theoretical

Trainee will be able to:

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

Example assessment question: How often are the pipettes calibrated?

6.1.2 Practical

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

Weigh varying quantities of substances on an analytical balance.

Choose proper pipettes and tips appropriate for volume to be measured and demonstrate proper technique in sample uptake, dispensing, and tip ejection. Trainee will be able to clean pipettes.

Demonstrate proper technique in loading and running centrifuges and dismantle the centrifuge for cleaning and disinfection.

Become familiar with the incubators and water baths, their operation and applications, checking and changing temperatures and routine cleaning.

Perform general cleaning on microscopes and dial between objectives.

Complete relevant documentation pertaining to equipment use, quality control checks and calibrations.

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Example assessment question: Trainee will be observed pipetting a series of liquid volumes.

6.2 OUTLINE

() refer to associated references

- I. Introduction to reading and reference materials **(1), (2)**
- II. Orientation of trainee to location of available equipment and equipment maintenance and calibration log book(s) in the laboratory
- III. Adjustable Pipettes **(2)**
 - A. Instruction on choice of size and operation of different pipets
 - B. Maintenance and cleaning
 - C. QC specifications, calibration and documentation **(5)**
- IV. Analytical Balances **(2)**
 - A. Instructions on operation and cleaning
 - B. QC specifications, calibration and documentation
- V. Water baths/Incubators
 - A. General serological water baths/incubators
 - B. DNA dedicated water baths **(2)**
- VI. Microscopes
 - A. Types and uses of microscopes in the laboratory **(4)**
 - 1. Polarizing/phase contrast microscope
 - 2. Stereoscope
 - 3. Comparison scope
 - B. General care and maintenance of microscopes **(3)**
 - C. Microscope adjustment – modified Kohler illumination **(3)**
- VII. Refrigerators/Freezers **(2)**
 - A. Location and storage application
 - B. Temperature specifications and alarm device
 - C. Cleaning (routine and major)
 - D. QC documentation **(5)**
- VIII. Centrifuges (microcentrifuge) **(2)**
 - A. Instruction on operation and cleaning (disinfection)
 - B. QC specifications, calibration and documentation **(5)**
 - 1. RPM
- IX. Thermometers (standard and digital) **(2)**
 - A. Instructions on handling, reading, and maintenance
 - B. QC specifications, calibration and documentation **(5)**
 - C. NIST traceable documentation
- XII. Laminar Flow Biological Safety Cabinet **(2)**
 - A. Principles of operation
 - B. Uses of laminar flow hoods
 - C. Certification and maintenance **(5)**

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6.3 THEORY

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 insure its reliability. Proper documentation of equipment calibrations and maintenance is crucial to demonstrating quality control in the laboratory. In addition, maintenance and calibration of equipment is an essential criteria outlined in the American Society of Crime Laboratory Directors Laboratory Accreditation Board manual. 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 following modules.

6.3.1 Readings

- (1) Austin Police Department Forensic Science Division Operations Manual
- (2) Austin Police Department Serology/DNA Unit SOP
- (3) General Care and Maintenance of Microscopes. From FBI Hair & Fiber Course Notebook. (attachment)
- (4) Saferstein, Richard. 1982. Forensic Science Handbook. Prentice-Hall, Inc. Pp. 416-528.
- (5) Laboratory Equipment Maintenance and Calibration Log Book(s) or equivalent

6.4 PRACTICE

6.4.1 Safety

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

6.4.2 Quality Control

Calibration schedule of equipment; maintenance and calibration forms and log books; documentation requirements.

6.4.3 Basic Skills

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Trainer will demonstrate proper technique for operating laboratory equipment. Trainer will give instructions on documentation of quality control procedures and calibration of pertinent equipment. Trainer will then observe performance of operations and documentation as trainee completes the assigned exercises:

- (1) Weigh out 5.34 grams of a substance and dissolve in water.
- (2) Pipet the following volumes: 5.2 ul, 10.0 ul, 20 ul, 50 ul, 190 ul, 205 ul, 881 ul into individual microcentrifuge tubes.
- (3) Demonstrate the procedure for centrifuging 2 microcentrifuge tubes for 15 seconds; for centrifuging 7 tubes for 3 minutes at 5000 rpm.

6.5 Written test

None

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Module 7 EVIDENCE HANDLING

Duration 1/2 to 1 day

Purpose To orient and acquaint trainee with evidence security, seals, tracking and documentation.

To enable a trainee to protect and store biological evidence.

7.1 OBJECTIVES

7.1.1 Theoretical

Trainee will be able to:

Discuss the security and limited access to the laboratory.

Describe an appropriate evidence seal.

Distinguish between long term and short term storage.

Describe the process of biological evidence storage.

Describe precautions taken to prevent contamination during evidence examinations.

Example assessment question: Why is evidence chain of custody important?

7.1.2 Practical

Trainee will be able to:

Receive, seal, and transfer evidence.

Document the chain of custody.

Properly package and store biological evidence.

Take precautions to prevent contamination, loss, and change of evidence.

Example assessment: Trainee will be observed sealing different evidence containers.

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7.2 OUTLINE

- () refer to associated references
- I. Evidence integrity and security **(1) (2) (4) (9, article 43)**
 - A. Proper storage and security of evidence
 - 1. Limited access
 - 2. Long term storage
 - 3. Short term storage
 - 4. Evidence seal requirements
 - 5. Protection from theft or loss
 - 6. Protection from deleterious change or contamination
 - 7. Biological evidence, refrigeration and drying
 - B. Chain of custody within the laboratory **(3) (4) (5)**
 - 1. Important issues
 - a) Keeping the chain
 - b) Good record keeping
 - 2. Receiving evidence **(1)**
 - a) Methods of receipt
 - b) Proper packaging of biological evidence
 - c) Case file documentation
 - d) Inventory evidence
 - e) Unique laboratory case number
 - f) Barcoding outer containers **(1)**
 - g) Proper evidence seals, tape and initials **(2)**
 - h) Computer data entry
 - 3. Outside container(s) identification
 - a) By the officer or agency
 - b) By the evidence tech or crime scene tech
 - c) By the analyst
 - 4. Identifying inside container(s) and evidence
 - a) Unique laboratory case number
 - b) Initials of analyst
 - c) Proper seals on containers
 - 5. Transfer of evidence
 - a) Intra-laboratory, barcoding **(1)**
 - 6. Disposition of evidence **(1)**
 - a) Methods of return
 - b) Case file documentation
 - c) Computer data entry, barcoding
 - II. Prevention of contamination and loss during evidence exams **(6) (7) (9, article #43)**
 - A. Laboratory layout
 - B. Case evaluation
 - C. Testing hierarchy **(9, articles #40, 41, 42)**
 - D. Precautions during evidence searches and analysis
 - 1. Single case/sample handling

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2. Separating evidence from reference samples
3. Preparing work surface areas
4. Frequent glove changes
5. Cleansing tweezers, scissors and tools

7.3 THEORY

The safe keeping of evidence and the chain of custody are crucial factors for the integrity and admissibility of evidence in a court of law. Good record keeping and documentation of evidence tracking within the laboratory is an essential criteria outlined in the American Society of Crime Laboratory Directors, Laboratory Accreditation Board manual. The precautions taken to prevent contamination, loss, and deleterious change to biological evidence is of utmost importance during storage and examinations and is another essential criteria.

7.3.1 Readings

- (1) Forensic Science Division SOP
- (2) American Society of Laboratory Directors / Laboratory Accreditation Board Manual,
- (3) Saferstein, Richard. 1982. Forensic Science Handbook. Prentice-Hall, Inc. Pp. 22-23.
- (4) Kirk, Paul L. and Lowell W. Bradford. 1972. The Crime Laboratory, Organization and Operation. 2nd printing. Charles C. Thomas, Publisher. Pp. 85-90, 94-98.
- (5) Moenssens, Andre A. et. al. 1973. Scientific Evidence in Criminal Cases. The Foundation Press, Inc. Pp. 18-19.
- (6) Saferstein, Richard. 2001. Criminalistics: An Introduction to Forensic Science, 6th ed. Prentice Hall, Inc. Pp. 381-385.
- (7) Serology/DNA SOP
- (8) DNA Court Book 1:

article #40, "DNA typing by PCR from Luminol Treated Latent Blood Traces."

article #41, "The "The Effects of Specific Latent Fingerprint and QD Examinations on the Amplification and Typing of the HLA DQalpha Gene Region in Forensic Casework", 1993.

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article #42, "Effects of argon laser lights, alternate light Source and Cyanoacrylate Fuming on DNA Typing of Human Bloodstains."

article #43, "Guidelines for the Collection and Preservation of DNA Evidence," Lee, et. al, 1991.

7.4 PRACTICE

7.4.1 Safety

Wear lab coat, mask, and gloves when working in the laboratory.

7.4.2 Basic Skills

No basic skills required.

7.4.3 Supervised Performance

The trainer will:

Discuss the security of evidence in the laboratory, by limiting access, secure storage areas, and evidence seals.

Demonstrate proper techniques of sealing evidence.

Give instruction on the documentation of receiving and returning evidence using the chain of custody.

Demonstrate the use of barcoding.

Discuss the inventory and intralaboratory transfer of evidence.

Discuss the necessary precautions taken to prevent contamination during evidence searches and analysis.

Discuss marking for identification and individual packaging of items.

Discuss the storage of biological evidence.

The trainee will:

Observe evidence being received, transferred, and returned

Demonstrate proper sealing of an envelope, paper bag, and a cardboard box.

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7.5 CONCLUSION

Written test

A written test will be given upon completion of module readings and exercises.

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Module 8 BLOOD IDENTIFICATION

Duration 2 to 4 weeks

Purpose Familiarize the trainee with blood evidence screening and presumptive tests.

8.1 OBJECTIVES

8.1.1 Theoretical

For the blood presumptive tests in the SOP, the trainee will be able to:

Name and describe the procedure for conducting each test.

Recognize the underlying chemical reaction for each test.

Identify two major factors that insure the reliability of the test.

Name the major categories of false positive reactions.

Describe the role and use of control samples.

Example assessment: What is the general chemical reaction that is the basis for each of our blood presumptive tests?

8.1.2 Practical

For the blood presumptive tests in the SOP and in use in the laboratory, the trainee will be able to:

Recognize potential bloodstains patterns, and distinguish between the variety of bloodstain patterns: transfer, drop, medium velocity, high velocity, etc.

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

Sampling and sample selection policies

Prepare a working solution of tetramethylbenzidine or describe the general procedure and underlying principle

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Describe the general procedure and underlying principle of the phenolphthalein test.

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

Properly document analyses and results.

Prepare reporting statements based on presumptive test results.

8.2 OUTLINE

- I. Blood screening
 - A. Blood **(3; pp. 103-114)**
 - 1. Composition and function of blood and blood components
 - 2. Properties of hemoglobin
 - 3. Location and appearance of bloodstains on evidence **(1;pp. 379-385)**
 - a) Basic bloodstain pattern interpretation **(5)**
 - 1) Patterns
 - 2) Documentation
 - 3) Limitations
 - b) Effects of degradation and aging on blood and bloodstains
 - 4. Blood in criminal evidence
 - a) Purpose for identification
 - b) Legal aspects
 - c) Limitations
 - 5. Collection and preservation
 - B. Presumptive testing **(6) (7) (8) (9) (10) (12) (13)**
 - 1. History and general theory of the testing procedures **(3;pp. 103-114)**
 - 2. Tests – use, chemical basis, sensitivity, stability, and specificity
 - a) Tetramethylbenzidine (TMB)**(4)**
 - b) Phenolphthalin (PHT)**(4)**
 - 3. Quality control
 - a) False positives
 - 1) Chemical oxidants
 - 2) Catalysts
 - 3) Plant peroxidases
 - b) Controls
 - 1) Reagent controls

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- III. Blood screening in casework
 - A. Case documentation **(4)**
 - B. Trace evidence collection
 - C. Location, identification, and documentation of bloodstains (sample selection)
 - D. Collection and preservation of evidentiary and reference samples
 - E. Counting and reporting examinations **(4)**
 - F. Reporting statements **(4)**

8.3 THEORY

Recognition of potential bloodstains is essential to the work of a forensic serologist. The presumptive tests are tools for recognizing bloodstains, and their results lay the foundation for determining the need for further testing. The presumptive tests provide basic information about a stain that may indicate the direction of further testing and eventually establish the importance of the evidence in court.

8.3.1 Limitations

Body fluids other than blood (perspiration, saliva, semen and urine) do not react positively to the presumptive tests. Because these tests depend on the oxidation of the substrate compound, a false positive reaction may occur in the presence of catalase, peroxidase (typically plant origin), cytochromes, strong oxidizing agents, and metallic salts. Color may develop (TMB, PHT) for a negative sample exposed to air for an extended period of time. If observation of the final result is delayed more than a few seconds, the final result will be compared to a negative control that was allowed to develop for the same length of time.

8.3.2 Readings

- (1) Saferstein, Richard. 2001. Criminalistics: An Introduction to Forensic Science, 6th edition. Pp. 327-330, 379-385.
- (2) Saferstein, Richard. 1982. Forensic Science Handbook, vol. 1. Pp. 267-323.
- (3) Gaensslen, R.E. 1983. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Department of Justice. Pp. 103-114, 221-227.
- (4) Austin Police Department Serology/DNA SOP
- (5) Bevel, Tom and Gardner, Ross. 1997. Bloodstain Pattern Analysis. Chapters 3 (49-75), 7 (145-168), and 8 (171-195).

Blood presumptive tests:

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- (6) Blake and Dillon. 1973. "Microorganisms and the presumptive tests for blood". Journal of Police Science and Administration 1(4): 395-400.
- (7) Garner, D. D. et al. 1976. "An evaluation of tetramethylbenzidine as a presumptive test for blood". Journal of Forensic Sciences. 21(4): 816-821.
- (8) Lytle & Hedgecock. 1978. "Chemiluminescence in the visualization of forensic bloodstains". Journal of Forensic Sciences 23(3): 550-562.
- (9) Laux. 1991. "Effects of luminol on the subsequent analysis of bloodstains". Journal of Forensic Science 36(5): 1512-1520.
- (10) Olsen, Robert Sr. 1986. "Sensitivity comparison of blood enhancement techniques". Identification News. August 1986. Pp. 5-6.
- (11) Milton, Cox. 1991. "A Study of the Sensitivity and Specificity of Four presumptive Tests for Blood". Journal of Forensic Sciences 36(5): 1503-1511.
- (12) Milton, Cox. 1990. "Effect of fabric washing on the presumptive identification of bloodstains". Journal of Forensic Sciences 35(6): 1335-1341.
- (13) Castelló Ponce, Ana and Fernando A. Verdú Pascual. 1999. "Critical revision of presumptive tests for bloodstains". Forensic Science Communications. 1(2) (<http://www.fbi.gov/programs/lab/fsc/>)

8.4 PRACTICE

8.4.1 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 liquid body fluids. Chemicals used may be carcinogenic or caustic. Blood may contain infective agents. Use universal precautions during evidence handling.

8.4.2 Equipment

balance, pipettes

Refer to Module 6, Equipment Use and Quality Control.

8.4.3 Reagent preparation

Tetramethylbenzidine

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8.4.4 Training samples

Various samples will be tested for presumptive blood.

8.4.5 Quality control skills

On the day of use, test blood presumptive reagents against a positive and negative control and record results on the Serology Worksheet.

8.4.6 Supervised performance

The trainee must be trained in each test available in the laboratory where casework is to be performed. The trainer must be present to demonstrate and observe each technique as well as to discuss findings, documentation, and safety issues.

I. Tetramethylbenzidine (TMB) presumptive test

Trainee will prepare the TMB reagent.

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

Trainer and trainee will discuss safety, interpretation and limitations of the test.

II. Phenolphthalin (PHT) presumptive test

Trainee will test a portion of each of the training samples with the PHT reagent.

Trainer and trainee will discuss safety, interpretation and limitations of the test.

III. Blood evidence processing

Trainer will demonstrate the recognition and documentation of blood evidence typical in casework. Actual cases may be used. A variety of bloodstain patterns and ages should be represented. Collection methods including cutting and swabbing will be demonstrated and documented as appropriate. Proper preservation techniques will be discussed and demonstrated. Substrate controls will be discussed. Presumptive blood tests will be conducted as appropriate and documented as for casework.

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Under direct observation of the trainer, trainee will demonstrate recognition and documentation of blood evidence typical in casework. Collection methods including cutting, scraping, and swabbing will be demonstrated and documented as appropriate. Proper preservation techniques will be demonstrated. Presumptive blood tests will be conducted as appropriate and documented as for casework.

8.4.7 Independent exercises

Trainee will independently test a minimum of five competency samples using a presumptive blood test. Documentation will be maintained in the trainee's competency notebook for review prior to independent casework.

8.5 CONCLUSION

Written test

The trainer will administer a written test.

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MODULE 9 SEMEN - IDENTIFICATION AND DETERMINATION

Duration 2 to 4 weeks

Purpose Familiarize the trainee with semen evidence screening, presumptive tests, and confirmatory tests.

9.1 OBJECTIVES

9.1.1 Theoretical

The trainee will be able to describe the components of semen and vaginal fluids and their functions.

For screening of semen evidence, the trainee will be able to:

Describe methods of locating semen stains on various substrates.

Give advantages and disadvantages of different methods of locating semen stains.

Describe the appearance of various stains under ultraviolet (or alternate or intense) light.

List the typical components of a sexual assault kit for female victims, male victims, and suspects.

For the semen presumptive tests in the SOP, the trainee will be able to:

Name and describe the procedure for conducting the acid phosphatase test.

Recognize the underlying chemical reaction for the acid phosphatase test.

Describe and explain the purpose of acid phosphatase mapping.

Identify the major factors that insure the reliability of the acid phosphatase test.

Name the major categories of false positive reactions.

Explain the need for confirmatory testing.

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Describe the role and use of control samples.

For the P30 tests in the SOP, the trainee will be able to:

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Name and describe the procedure for conducting the test.

List the advantages and limitations of the test.

Recognize the underlying chemical reaction for the test.

Identify the major factors that insure reliability of the test.

For microscopic identification of spermatozoa, the trainee will be able to:

Identify each part of a normal stained spermatozoa.

Identify each part of a normal stained epithelial cell.

Identify material in a stained slide as non-sperm or non-epithelial as appropriate.

Name and describe the procedure for staining a microscope slide for identification of spermatozoa.

Example assessment: Name at least three sources of acid phosphatase.

9.1.2 Practical

The trainee will be able to:

Recognize possible semen stains on a variety of substrates using visual screening, an ultraviolet (or alternate or intense) light source, and acid phosphatase mapping techniques.

Properly collect a portion of a possible semen stain for testing and storage.

Prepare a working solution of AP spot.

Describe the general procedure and underlying principle of the nuclear fast red and picroindigocarmine stains.

Conduct and interpret each semen confirmatory test used in the laboratory.

Sample selection policy: determination of stains to test and collect

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Properly document analyses and results.
Prepare reporting statements based on presumptive and confirmatory test results.

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Example assessment: Extract and identify spermatozoa from a deposit of dilute semen on a buccal swab sample.

9.2 Outline

() refer to associated references

- I. Biological aspects of the male and female reproductive systems (4), (7)
 - A. Vaginal fluid
 1. Biochemistry and function
 2. Vaginal fluid components
 - a) chemical
 - 1)acid phosphatase
 - b) cellular
 - 1)epithelial cells
 - 2)yeast
 - 3)bacteria
 - 4)blood cells (red and white)
 - 5)protozoa
 - B. Semen
 1. Biochemistry and function
 2. Identification of semen components [\(2;section 10.13-10.14\)](#)
 - a) chemical components
 - 1)acid phosphatase
 - 2)spermine
 - 3)choline
 - 4)p30
 - b) spermatozoa
 3. Persistence of semen components **(5), (6), (18)**
 - a) in vaginal vault
 - b) in anus and rectum
 - c) in oral cavity
 - d) in stains
- II. Semen in criminal evidence **(8), (9), (10)**
 - A. Purpose for identification
 - B. Legal aspects
 - C. Limitations
- III. Semen screening
 - A. Locating semen stains [\(1;pp. 349-350\)](#)
 1. Visual examination
 - a) appearance

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- b) limitations
- 2. Light sources (UV, alternate) [\(2;section 10.12\), \(3\)](#)
 - a) appearance
 - b) limitations
- B. Collection and preservation
- C. Presumptive testing [\(1;pp. 348-352\)](#)
 - 1. History and general theory of the testing procedures
 - 2. Tests – use, chemical basis, sensitivity, stability, and specificity
 - a) acid phosphatase **(3)**
 - 1) spot test
 - 2) mapping
 - 3. Quality control
 - a) False positives
 - b) Controls
 - 1) Reagent controls – use and interpretation
- D. Confirmatory tests
 - 1. Sample extraction methods **(1), (3)**
 - 2. Identification of spermatozoa **(3), (11), (12)**
 - a) microscopy review
 - 1) phase contrast microscopy
 - 2) light microscopy
 - b) staining techniques **(16)**
 - c) sperm morphology
 - 1) human
 - 2) other species
 - d) morphology of other common components
 - 1) bacteria
 - 2) yeast
 - 3) protozoa
 - 4) epithelial cells
 - 5) semen mixtures
 - a. semen and feces
 - b. semen and saliva
 - 3. Identification of p30 **(3)**
 - a) ABACard test strips **(13), (14)**
- E. Semen screening in casework
 - 1. Sexual assault evidence **(3)**
 - a) sexual assault kits
 - 1) female victim
 - 2) male victim
 - 3) suspect kit
 - b) clothing, bedding, miscellaneous evidence
 - 2. Case documentation **(3)**
 - 3. Trace evidence collection

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4. Location, identification, and documentation of semen evidence; sample selection **(3)**
5. Collection and preservation of evidentiary and reference samples **(3)**
6. Counting and reporting examinations
7. Reporting statements **(3)**

9.3 Theory

The identification of semen and semen stains is of major importance in criminal offenses such as homicide and sexual assault. 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 provide incontrovertible proof of the sexual nature of the perpetrator's contact. Information about the presence, location, and state of degradation of semen can corroborate a victim's statement or provide a basis for further investigation. Unlike blood testing in our laboratory, presumptive tests for semen can generally be confirmed through further testing. Identification of semen is typically followed by separation of and DNA typing of sperm and non-sperm cells to eventually associate a stain with its source of origin.

9.3.1 Considerations

Direct, intense light will degrade DNA over time. Limit exposure of stains to ALS light.

Fluorescence of semen stains is thought to be due to the conversion of non-protein substances into compounds capable of fluorescence as well as the growth of the fluorescent bacterium *Pseudomonas fluorescens* in the stain. Therefore, older stains may fluoresce more efficiently than newer stains.

Urine stains are also capable of fluorescence, though it is usually less intense than for semen stains.

The phosphatase activity of stains has been observed to be more concentrated in the periphery of a stain, whereas the sperm cells tend to concentrate more in the center. Acid phosphatase activity can be detected 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. Acid phosphatase can be detected in vaginal aspirates and swabs taken from a living victim as long as 24 hours after coitus. 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.

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A positive result on an AP spot test may be due to acid phosphatase from another body fluid. The level of acid phosphatase in the blood of men with prostatic cancer increases dramatically. 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. Some vaginal deodorants and spermicides produce a false positive reaction. Contact with light and air will cause the AP Test Reagent to turn purple even in the absence of acid phosphatase; the reaction must be interpreted within 60 seconds of application of the AP Test Reagent.

Only the prostatic secretions of humans and monkeys contain large acid phosphatase concentrations. 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. A few chemicals that could be found as components of vaginal deodorants or spermicidal contraceptives inhibit acid phosphatase. The AP test can be negative even when sperm are present.

Survival time of sperm in the vagina is affected by number of sperm present in the ejaculate as well as the conditions in the vagina, including menstrual cycle stage. Sperm survive longer in the cervix than in the vagina, up to 17 days as compared to up to 6 days for recovery of non-motile sperm. However, the persistence of sperm declines dramatically after about 48 hours. Sperm have been found on anal and rectal swabs taken up to 24 and 20 hours respectively following a sexual assault. In a non-living victim, sperm have been recovered 16 days after death.

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

Semen of normal, fertile men can contain almost 40% abnormal forms of spermatozoa, generally with elongated, thin, or irregular head shapes or bent or coiled tails.

Fibers and certain bacteria and molds 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.

Dry semen stains stored at -20°C, 4°C, or room temperature show little loss of p30 over 5 months.

The false negative "high dose hook effect" occurs when p30 concentration in the sample is so high that only some of the p30 molecules are bound by mobile antibodies and 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

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antibody-antigen complex, which is necessary for visualization of the dye. This method is not expected to detect a semen dilution below 1:1,000,000. A dilution of 1:809,200 was barely detectable during validation.

A positive result may be due to p30 from another male body fluid. 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. Vasectomy does not affect the amount of p30 secreted into the seminal fluid. No female tissues or fluids have been shown to exhibit p30 activity. Orangutan and macaque semen contains p30 at concentrations within human range but relatively very low acid phosphatase activity.

9.3.2 Readings

Comprehensive:

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- (1) Baechtel, Samuel F. 1988. The identification and individualization of semen stains. *In: Forensic Science Handbook, Volume 2.* Richard Saferstein, ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. Chapter 7, pp. 347-369.
- (2) Gaensslen, Robert E. 1983. Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Department of Justice, National Institute of Justice. Sections 10.1-10.4, 10.12, 10.14, 11.6, 12.1, 14.
- (3) Austin Police Department [Serology/DNA](#) SOP

Biological aspects of the male and female reproductive systems:

- (4) Brown, Barry L. 1983. Anatomy, physiology and biochemistry of the female reproductive system. *In: Proceedings of a forensic science symposium on the analysis of sexual assault evidence.* Federal Bureau of Investigation. Quantico, Virginia. pp. 3-19.
- (5) Davies, Anne and Elizabeth Wilson. 1974. The persistence of seminal constituents in the human vagina. *Forensic Science* 3: 45-55.
- (6) McCloskey, K. L., G. C. Muscillo, and B. Noordewier. 1975. Prostatic acid phosphatase activity in the postcoital vagina. *Journal of Forensic Sciences* 20(4): 630-636.
- (7) Sherins, Richard J. and Barry L. Brown. 1983. Anatomy, physiology and disorders of the male reproductive system. *In: Proceedings of a forensic science symposium on the analysis of sexual assault evidence.* Federal Bureau of Investigation. Quantico, Virginia. pp. 21-43.

Semen in criminal evidence:

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Approved by: Laboratory Director

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- (8) Enos, W. F. and J. C. Beyer. 1978. Spermatozoa in the anal canal and rectum and in the oral cavity of female rape victims. *Journal of Forensic Sciences* 23(1): 231-233.
- (9) Tanton, Richard L. 1983. Interpretation of results in sexual assault cases and reporting methods: A serology report writing exercise. *In: Proceedings of a forensic science symposium on the analysis of sexual assault evidence.* Federal Bureau of Investigation. Quantico, Virginia.
- (10) Texas Commission on Law Enforcement Officer Standards and Education. 1998. Sexual Assault. Chapters 4 and 7. Appendices D and F. Web publication: <http://www.utexas.edu/cee/dec/tcleose/assault/chap1.html>.

Identification of spermatozoa:

- (11) Chang, Thomas S. K. 1983. Seminal cytology. *In: Proceedings of a forensic science symposium on the analysis of sexual assault evidence.* Federal Bureau of Investigation. Quantico, Virginia. pp. 45-56.
- (12) Willott, G. M. 1982. Frequency of azoospermia. *Forensic Science International* 20:9-10.

Identification of p30:

- (13) Abacus Diagnostics. 1999. OneStep ABACard p30 test for the forensic identification of semen. Technical information sheet for catalog #308332. Abacus Diagnostics, West Hills, CA. pp. 1-2.
- (14) Benton, K., J. Donahue, and M. Valadez, Jr. 1998. Analysis of the OneStep ABACard PSA test for use in the forensic laboratory. Texas Department of Public Safety Crime Laboratory Service. Unpublished. pp. 1-8. Carradine, C. 1998. The Evaluation of the Onestep ABACard Prostate Specific Antigen (p30) Test for the Forensic Identification of Semen. Unpublished. pp 1-7.
- (15) Baechtel, F. S. 1983. Immunological methods for seminal fluid identification. *In: Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence.* Washington, D.C.: FBI Laboratory. Page 83.

Semen screening in casework:

- (16) Gaensslen, R. E., Henry C. Lee, Jack Mertens, and Mark D. Stolorow. 1983. Workshop I: Staining and extraction techniques. *In: Proceedings of a forensic science symposium on the analysis of sexual assault evidence.* Federal Bureau of Investigation. Quantico, Virginia. pp. 135-144.

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- (17) The Office of the Attorney General. Sexual Assault Prevention and Crisis Services Division. 1998. Texas Evidence Collection Protocol.
- (18) Willott, G. M. and J. E. Allard. 1982. Spermatozoa – Their persistence after sexual intercourse. Forensic Science International 19:135-154.

Safety:

Material Safety Data Sheets

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9.3.3 Problems/Exercises

None

9.4 Practice

9.4.1 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 liquid body fluids. Chemicals used may be carcinogenic or caustic. Blood may contain infective agents. Use universal precautions during evidence handling.

Avoid looking directly into the alternate light source. Follow safety and use directions provided with the instrument.

9.4.2 Equipment

60°C dry oven, alternate light source, balance, microcentrifuge, microscope, refrigerator, stir plate. Refer to Module 6, Equipment Use and Quality Control.

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9.4.3 Reagent preparation

ABAcad p30 test: purchased from Abacus Diagnostics

Acid phosphatase test: AP spot

Christmas tree stain: nuclear fast red solution, picroindigocarmine solution

9.4.4. Training samples

[Known semen stains of varying concentrations, bloodstains, vaginal fluid stains, urine stains, sweat stains, and milk stains.](#)

[5 unknown and/or mixed stains on different fabrics.](#)

9.4.5 Basic Skills

Microscope use.

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Alternate light source.

9.4.6. Quality control skills

On the day of use, test acid phosphatase spot test solution reagent against a positive and negative control and record results on the Serology Worksheet.

9.4.7. Supervised performance

The trainee must be trained in each test available in the laboratory where casework is to be performed. The trainer must be present to demonstrate and observe each technique as well as to discuss findings, documentation, and safety issues.

I. Location of semen stains

Trainer and trainee will discuss appearance of a variety of body fluid stains on various substrates.

Trainer and trainee will discuss safety, interpretation and limitations of using an alternate light source.

Trainer will demonstrate use of an alternate light source.

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

II. Acid phosphatase (AP) spot test

Trainee will prepare the AP spot test reagent.

Trainee will test a portion of each of the training samples with the AP spot test reagent.

Trainer and trainee will discuss safety, interpretation and limitations of the test.

III. Acid phosphatase (AP) mapping

Trainee will test selected training samples on various cloth substrates with the AP mapping test.

Trainer and trainee will discuss safety, interpretation and limitations of the test.

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IV. Stain extraction

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Trainee will extract a portion of selected training samples

Trainer and trainee will discuss appropriate use and limitations of each extraction technique.

V. ABACard test for p30

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

Trainer and trainee will discuss limitations of the test, including potential false negative reactions.

VI. Identification of spermatozoa

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Trainer and trainee will discuss morphology of various cellular and acellular components in typical microscope slide preparations for sperm identification.

Trainee will stain the training smears.

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Trainee will examine the stained training smears using normal light microscopy at the full range of magnification available.

VII. Semen screening in casework

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Trainee will observe the analysis of at least five typical sexual assault cases including casework documentation; preparation of reference blood, saliva, and semen standards for preservation; analysis of evidence for the presence of semen (location, presumptive, and confirmatory tests); and the collection and preservation of evidentiary stains.

Under direct observation of the trainer, trainee will demonstrate analysis of semen evidence typical in casework including casework documentation; collection of trace evidence; preparation of standards; analysis for the presence of semen; and collection and preservation of evidentiary stains and substrate controls.

VIII. Independent exercises

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Trainee will independently test a minimum of five competency samples for the presence of semen. Documentation will be maintained in the trainee's competency notebook for review prior to independent casework.

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At the discretion of the trainer, this competency battery may be administered prior to the supervised exercise in Semen Screening in Casework.

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9.5 Conclusion

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Written test

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A written test will be administered by the trainer.

Deleted: examination

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Module 10 TRACE EVIDENCE COLLECTION

Duration 1 to 2 days

Purpose Familiarize the trainee with the proper methods of collection and preservation of trace evidence.

10.1 Objective

10.1.1 Theoretical

Trainee will be able to:

Describe trace evidence.

Recognize trace evidence in laboratory situations.

Understand the importance and significance of the collection and preservation of trace evidence.

Understand trace evidence procedures of the APD laboratory.

10.1.2. Practical

None

10.2 OUTLINE

() refer to associated references

- I. Introduction
 - A. Principles of trace evidence examination
 - 1. Transfer and persistence of trace evidence
 - a) Locard's exchange principle
 - b) Persistence
 - B. Identification vs. comparison **(1)**
- II. Evidence recognition, collection and handling techniques **(2)**
 - A. Recognition/detection
 - 1. Visual search
 - a) General
 - b) Oblique lighting
 - c) Alternative light sources
 - d) Magnification
 - B. Collection
 - 1. Picking
 - a) Case evaluation
 - b) Documentation

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- c) Technique
- d) Storage
- 2. Tape lifting
 - a) Case evaluation
 - b) Documentation
 - c) Technique
 - d) Storage
 - e) Examination
 - f) Recovery

10.3 THEORY

10.3.1 Readings

- (1) Technical Working Groups of Material Analysis, Trace Evidence Recovery Guideline, Federal Bureau of Investigation, 1998.
- (2) Serology/DNA SOP.

10.4 PRACTICE

None.

10.5 CONCLUSION

10.5.1. Written test

None.

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Module 11 REPORT WRITING AND CASE REVIEW I

Duration 1 to 2 days

Purpose To provide guidelines for Serology casework report writing and for technical and administrative case reviews.

11.1 OBJECTIVES

11.1.1. Theoretical

Trainee will be able to write clear, concise reports that are provided to the submitting officer, prosecuting and defense attorneys.

Trainee will be able to perform technical case reviews for Serology casework.

Example assessment question: What percent of cases are required to have a technical review?

11.1.2. Practical

Trainee will be able to reduce technical data to a concise conclusion.

Trainee will be able to technically review a Serology case from the testing performed to the written report.

Trainee will be able to produce a report in a format easily understandable to a non-scientist.

Example assessment question: List five areas of general information that must be included in the report.

11.2 OUTLINE

() refer to associated references

- I. Report writing
 - A. General information
 - 1. Additional submissions
 - 2. Case documentation
 - a) Documentation, technical notes, case notes
(1)
 - b) Policy **(1)**

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- c) Case related correspondence **(1)**
 - 3. Records **(1)**
 - 4. Laboratory Reports **(2), (3;standard 11.1)**
 - a) Elements in reports **(2)**
 - b) Format of reports **(2)**
 - B. Evidence analyzed - all evidence requested should be listed.
 - C. Requested analysis - this section can be a mixture of the laboratory's normally used statement and any special requests made by the officer.
 - D. Results of analysis
 - 1. Blood examinations **(2)**
 - 2. Semen examinations **(2)**
 - E. Conclusions - this portion of the report is optional for a screening letter. There will not normally be additional conclusions beyond general screening statements.
 - F. Investigative leads - 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.
 - G. Disposition - Identify items retained frozen in the laboratory and returned to property.
- II. Technical Review **(1), (3;standard 12.1)**
- A. The technical review is required. The case information should be organized in a logical fashion.
 - B. The conclusions should be written to ensure that results are communicated properly and clearly
- III. Administrative Review **(1), (3;standard 12.1)**

11.3 THEORY

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. 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.

11.3.1. Readings

- (1)** Forensic Science Division SOP

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- (2) Serology/DNA SOP and Training Manual

- (3) FBI, "Quality Assurance Standards for Forensic DNA Testing Laboratories"
Standard 11.1
Standard 12.1

11.3.2. Quality Control

I. Report writing

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. All results or conclusions in the formal report must be 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.

II. Technical review

The review is a thorough evaluation of the worksheets to determine what analyses were conducted, if the requested analyses were performed, what the results of those analyses were, if the conclusions reached were justified, and if the proper information was included in the report. A thorough review of appropriate quality control records is essential to the technical review.

11.3.3. Problems/exercises

The trainer will provide at least 5 cases involving general serology screening to the trainee to allow familiarity with the method of report writing in the laboratory.

11.4 PRACTICE

The trainee will write a results/conclusion paragraph and a disposition paragraph for the following raw data results:

- 1. A. blood sample from victim – not tested – blood card made
B. vaginal swabs - (+) AP
C. vaginal slides - (+) sperm

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2.
 - A. blood sample from victim – not tested – blood card made
 - B. vaginal swabs - (-) AP
 - C. vaginal slides - (-) sperm
 - D. panties - (-) AP

3.
 - A. swabs from counter top - (+) blood presumptive
 - B. swabs from floor - (+) blood presumptive
 - C. swabs from display case - (-) blood presumptive
 - D. blood from suspect – not tested – blood card made

4.
 - A. blood sample from victim – not tested – blood card made
 - B. suspect's shirt, stain 1 - (+) blood presumptive,
 - C. suspect's pants, stain 3 - (+) blood presumptive,

5.
 - A. victim's blood – not tested – blood card made
 - B. vaginal slides - (-) sperm
 - C. vaginal swabs - (+) AP, (+) p30
 - D. panties - (+) AP

11.5 CONCLUSION

11.5.1. Written test

None

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MODULE 12 **Law, Pretrial Preparation and Court Testimony for the Expert Witness**

Duration 7 days minimum for reading material and written assignments, court observations and mock trials will be on-going with other training

Purpose To familiarize the trainee with the basic legal terminology and applicable Texas Criminal Laws listed in the Penal Code, Code of Criminal Procedure and Rules of Criminal Evidence as well as APD policy and procedure regarding court testimony, motions of discovery and court orders. To prepare the trainee for court testimony as an expert witness.

12.1 **OBJECTIVES**

12.1.1 **Theoretical**

Trainee will become familiar with APD general policy as well as the policies and procedures of the APD Forensic Science Division governing expert testimony

Trainee will learn the basic legal terms frequently used in the laboratory system such as chain of custody, probable cause, voir dire, subpoena and others..

Trainee will become familiar with Article VII of the Rules of Criminal Evidence pertaining to opinions and expert testimony as well as the court cases, Daubert, Kelly, Frye and Hartman, that define expert testimony.

Trainee will learn proper dress and courtroom etiquette for an expert witness as well as some basic techniques on how to qualify as an expert witness in court and how to present scientific principles and test results to a judge and jury in a legally admissible and understandable manner.

Example assessment question: What is a Daubert hearing?

12.1.2. **Practical**

None.

12.2 **OUTLINE**

() refer to associated references

- I. Expert Testimony
 - A. APD general policy **(1)**

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- B. Policies and procedures of the APD Forensic Science Division **(2)**
- II. Basic legal terminology **(5)**
- III. Texas Criminal Laws - Penal Code **(3;chapter 1)**
- IV. Code of Criminal Procedure **(3;chapter 2)**
- V. Rules of Criminal Evidence **(3;chapter 3)**
- VI. Role of the Expert Witness **(4;pages 1-27)**
- VII. Courtroom Demeanor **(5), (6), (7)**
 - A. Pretrial preparation
 - B. Proper dress and appearance
 - C. Courtroom etiquette
 - D. Body language
 - E. Qualifying as an expert witness
 - F. Presentation of scientific principles and results in an understandable manner
 - G. Direct examination
 - H. Cross examination
 - I. Subpoenas, motions for discovery **(2)**
- VIII. Court rulings pertaining to scientific testimony **(13)**
 - A. Frye v. United States **(4;page 23)**
 - B. Barry Dean Kelley, Appellant, v. The State of Texas **(14)**
 - C. William Daubert v. Merrell Dow Pharmaceuticals, Inc. **(11), (12), (15)**
 - D. Allen Spock Hartmann, Appellant v. The State of Texas **(16)**
- IX. DNA specific court rulings
- X. Curriculum Vitae **(7)**

12.3 THEORY

Expert testimony in court is the most visible aspect of any forensic scientist's duties, especially in the age of court television. Therefore, a forensic scientist must be trained not only to use good scientific practices and proper documentation in his casework, but also to become the best witness possible in a court of law. This means learning to qualify as an expert witness under current case law and to present his/her information in easily understood terminology with a credible manner. An expert witness must also assist the prosecution in understanding the scientific procedures involved and in developing a strategy for presenting the expert's results and opinions to a judge and jury. The forensic scientist should know how to respond to motions and court orders from the defense and become familiar with common defense techniques. This module is designed to give the trainee an overview of Texas Criminal Law and APD policies and procedures regarding court testimony as well as practice and training in realistic court situations.

12.3.1 Readings

- (1) Austin Police Department Policy Manual

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- (2) Forensic Science Division SOP
- (3) Texas Criminal and Traffic Law Manual. 2001-2002 Revised Edition
 - Chapter 1. Penal Code
 - PC Chapter 12. Punishments
 - PC Chapter 19. Criminal Homicide
 - PC Chapter 20. Kidnapping and Unlawful Restraint
 - PC Chapter 21 . Sexual Offenses
 - PC Chapter 22. Assaultive Offenses
 - PC Chapter 36. Bribery and Corrupt Influence
 - PC Chapter 37. Perjury and Other Falsification
 - Chapter 2. Code of Criminal Procedure
 - CCP Chapter 12. Limitation
 - CCP Chapter 18. Search Warrants
 - CCP Chapter 20. Duties and Powers of the Grand Jury
 - CP Chapter 24. Subpoenas and Attachment
 - CCP Chapter 28. Motions, Pleadings and Exceptions
 - CCP Chapter 27. The Pleadings in Criminal Actions
 - CCP Chapter 38. Evidence in Criminal Actions
 - CCP Chapter 39. Depositions and Discovery
 - CCP Chapter 49. Inquests Upon Dead Bodies
 - CCP Chapter 57. Confidentiality of Identifying Information of Sex Offense Victims
 - Chapter 3. Rules of Criminal Evidence
 - CE Article IV. Relevancy and Its Limits
 - RCE Article VI. Witnesses
 - RCE Article VII. Opinions and Expert Testimony
 - RCE Article VIII. Hearsay
 - RCE Article X. Contents of Writing, Recordings and Photographs
- (4) Kuzmack, Nicholas T. 1982. Legal Aspects of Forensic Science. *In*: Forensic Science Handbook. Richard Safetstein, ed. Prentice-Hall, Inc . Chapter 1, pp. 1-23.
- (5) Glossary of Legal Terms, unknown source
- (6) Questionnaire for Interviewing Expert Witnesses
- (7) Expert Witness Curriculum Vitae. 1998. Expert Witness Handbook. Alcohol Testing Alliance
- (8) Dunn, R. Roger. 1991. Persuasive Expert Testimony. IAAI Attorney Advisory Committee. Dallas, TX

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- (9) Phillips, K. A. 1977. The "Nuts and Bolts" of Testifying as a Forensic Scientist. *Journal of Forensic Sciences* 22(2): 457-463.
- (10) Kogan, J. D. 1978. On Being a Good Expert Witness in a Criminal Case. *Journal of Forensic Sciences* 23(1): 190-200.
- (11) Thornton, John I. 1994. Courts of Law v. Courts of Science: A Forensic Scientist's Reaction to Daubert. *Shepard's Expert and Scientific Evidence Quarterly*. Vol. 1, Winter No. 3.
- (12) Questions During a Daubert Ruling, courtesy of Lorna Beasley. TX v. Charles Daniel Tuttle, 114th District Court, Smith County, Judge Cynthia Kent. February 22, 1996.
- (13) Case Law Concerning Expert Witness Testimony. 1998. *Expert Witness Handbook*. Alcohol Testing Alliance.
- (14) Barry Dean Kelley, Appellant v. The State of Texas. Appellee. copyright West Group. 1997
- (15) William Daubert v. Merrell Dow Pharmaceuticals, Inc. copyright West Group, 1997.
- (16) Allen Spock Hartman, Appellant v. The State of Texas. Appellee. copyright West Group. 1997
- (17) Inman, Keith and Norah Rudin. 1997. *An Introduction to Forensic DNA Analysis*. CRC Press. Chapter 11.

12.4 PRACTICE

12.4.1. Quality Control

The first court testimony of the trainee will be monitored

12.4.2. Demonstration by trainer and/or supervised performance

Trainee will observe testimony of more experienced Forensic Scientists. The trainee's performance in the mock trial sessions will be observed by the trainer. Trainer will also review the written exercises prepared by the trainee.

12.4.3. Independent exercises

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Observe courtroom testimony of other, more experienced experts on cases involving serology and/or DNA typing. Keep a record of witnesses and cases observed.

Interview three expert witnesses in serology and/or DNA typing,

Prepare first *Curriculum Vitae*. **(10)**

Participate as an expert witness in a mock trial consisting of prosecution questions and aggressive defense questions

12.5 CONCLUSION

Written test

A written test will be administered by the trainer.

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MODULE 13 INTRODUCTION TO DNA - HISTORY OF DNA

Duration 1 to 2 days

Purpose Enable a trainee to communicate with appropriate forensic DNA terms.

Educate a trainee on the earlier procedures and advances in forensic DNA.

13.1 OBJECTIVES

13.1.1. Theoretical

Define and understand basic genetic, heredity, and forensic DNA typing terms.

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

Describe the limitations of DNA analysis.

Example assessment question: Define alleles.

13.2 Practical

None.

13.3 OUTLINE

() Refer to associated references

- I. Introduction to forensic DNA **(1,3)**
 - A. Definition, importance, legal value
 - B. The scientific basis of DNA typing
 - C. Other areas utilizing DNA typing techniques
- II. Overview of forensic DNA typing systems **(2;chapter 6)**
 - A. RFLP
 - B. PCR - HLA Dq α and D1S80
 - C. PCR – STR
 - D. Mitochondrial DNA
- III. Procedures for forensic DNA analysis **(2;chapter 7)**
 - A. Isolation of DNA
 - B. Determining quality and quantity of DNA
 - C. PCR amplification
 - D. Analysis of PCR product

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IV. Human Identity Testing Chronology (4)

13.4 THEORY

The ability to understand and use proper biological terminology is necessary for successful communications in both the forensic field and in the courtroom. The history of the development and past methods in human identification testing will provide the trainee with a foundation of the concepts and the procedures used in the laboratory for forensic DNA testing.

13.4.1. Readings

- (1) Serology/DNA SOP.
- (2) Inman, Keith and Norah Rudin. 1997. An Introduction to Forensic DNA Analysis. CRC Press LLC. Chapters 6 & 7 .
- (3) National Research Council. 1995. The Evaluation of Forensic DNA Evidence. National Academy Press.
- (4) Human Identity Testing Chronology. Perkin Elmer. 7/23/96

13.4.2. Practice

None.

13.5 CONCLUSION

Written test

A written test will be administered by the trainer.

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MODULE 14 CONTAMINATION ISSUES

Duration 1 to 2 days

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

14.1 OBJECTIVES

14.1.1. Theoretical

Trainee will be able to:

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

Describe actions taken to preserve DNA.

Describe the possible sources of contamination in the laboratory.

Describe the necessary precautions taken to prevent contamination within each dedicated work area.

Discuss the contamination contingency plan.

Example assessment question: Discuss the precautions taken in the amplified DNA work areas.

14.1.2. Practical

Trainee will be able to:

Properly clean work surfaces.

Properly select the correct cleaning process and/or solution for DNA equipment and supplies, such as pipettes, microcentrifuges, water baths, reagent bottles and the thermal cycler.

Properly cleanse forceps, scissors and other tools.

Demonstrate proper handling of samples, handling of microcentrifuge tubes, proper pipetting techniques, and use of reagents.

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Properly dispose used or contaminated materials and supplies.

Example assessment question: Trainee will be observed cleaning and preparing a work surface in the DNA extraction area.

14.2 OUTLINE

() refer to associated references

- I. DNA degradation **(1) (2) (5) (8;75-78) (9)**
 - A. Chemicals, detergents
 - B. UV light, sunlight
 - C. Heat
 - D. Humidity
 - E. Bacteria, microorganisms
 - F. Endo and exonucleases
 - G. Biological fluid mixtures
 - H. Shear forces
 - I. Time
- II. Preserving stains / DNA **(4) (8;186-187)**
 - A. Drying
 - B. Refrigeration or freezing
 - C. EDTA additive
- III. Sources of laboratory contamination **(2)**
 - A. Human genomic DNA from the environment
 - B. Sample to sample contamination
 - C. PCR product carry-over
- IV. Contamination prevention measures **(6) (7) (9)**
 - A. Isolated and dedicated work areas
 - 1. Evidence handling work area
 - 2. DNA extraction work area
 - 3. PCR set-up work area
 - 4. Amplified DNA work area
 - 5. Dark room
 - B. Special precautions **(6)**
 - 1. Dedicated equipment and lab supplies
 - 2. Clean work surfaces and equipment
 - 3. Clean cutting surface
 - 4. Frequent glove changes
 - 5. Cleansed scissors or disposable blades, forceps
 - 6. Disposable pipette tips
 - 7.
 - 8. Avoiding splashes
 - 9. UV germicidal lamps
 - 10. Separating evidence from reference samples **(3)**
 - 11. Aliquot reagents
- V. Contamination Contingency Plan - Response **(6)**

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14.3 THEORY

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

14.3.1. Readings

- (1) Inman, Keith and Norah Rudin. 1997. An Introduction to Forensic DNA Analysis. CRC Press LLC. Pp. 11-17.
- (2) National Research Council. 1998. DNA Technology in Forensic Science. National Academy Press.
- (3) National Research Council. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press. Pp. 82-84.
- (4) DNA Court Book 1: Article #44, "Effects of Blood Storage Time and Temperature on DNA Yield and Quality," 1993.
- (5) Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. 1989. Federal Bureau of Investigation. Quantico, Virginia. Pp. 14-15, 25-26, 219-220, 257-261, 267-271.
- (6) Serology/DNA SOP
- (7) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual. Chapter 2.
- (8) Farley, Mark and James Harrington. 1991. Forensic DNA Technology. Lewis Publishers, Inc. Pp. 75-78, 186-187.
- (9) Reynold, et.al. 1991. Analysis of Genetic Markers in Forensic DNA Samples Using the Polymerase Chain Reaction. American Chemical Society.
- (10) Instruction manual for thermal cycler.

14.4 PRACTICE

14.4.1. Safety

Wear a lab coat, mask, and gloves when working in the laboratory.

14.4.2. Quality control

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

14.4.3. Reagent preparation

Trainee will review reagent preparation protocols in the SOP

14.4.4. Demonstration by trainer and/or supervised performance

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

14.4.5. Exercises/practice

1. Clean and prepare a work area in the extraction room.
2. Properly clean the nearest microcentrifuge with the appropriate cleaning solution.
3. Trainee will be given 5 samples, each inside a separate envelope. Trainee will label 5 microcentrifuge tubes (1 through 5) and place them in a rack. Trainee will be observed removing portions from each of the samples and placing them into the appropriate tubes. Trainee will be observed pipetting first 500 microliters into each tube, then pipet 10 microliters into each tube.

14.5 CONCLUSION

14.5.1. Written test

None.

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Module 15 STAIN EXTRACTION

Duration 1 to 2 weeks

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

15.1 OBJECTIVES

15.1.1. Theoretical

Trainee will:

Describe and become familiar with the various extraction methods and purification techniques used on forensic specimens.

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

Describe techniques to increase the yield of DNA and to remove inhibitors of PCR amplification.

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

Example assessment question: What are the benefits in using Qiagen?

15.1.2. Practical

Trainee will be able to:

Prepare and store the necessary reagents used in DNA extractions.

Perform the necessary precautions to prevent contamination during extraction procedures.

Isolate DNA using current extraction methods.

Perform the differential lysis extraction of semen stains.

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

15.2 OUTLINE

- () Refer to associated readings
- I. Isolating DNA by QIAamp: Manual and QIAcube **(1)**
 - A. Purpose, benefits
 - B. Methods
 - 1. Bloodstain extraction
 - 2. Buccal cell extraction
 - 3. Differential extraction
 - 4. Hair root extraction (optional)
- II. Maxwell 16 Extraction
- III. Swab Solution Extraction of reference samples
- IV. Special extractions and removing inhibitors **(3), (4)**
 - A. Purification of extracted DNA samples using QIAamp **(1)**
- V. Quality control **(5)**
 - A. Forms, worksheet documentation
 - B. Critical reagents
 - C. Reagent blanks
 - D. Separate extraction of known and questioned samples
- VI. Preserving extracted DNA

15.3 THEORY

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

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.

15.3.1. Readings

- (1) Serology/DNA Technical Manual
- (2) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual.Chapter 3.

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- (3) Shutler, Gary, et. al. 1999. Removal of a PCR Inhibitor and resolution of DNA STR Types in Mixed Human-Canine Stains from a Five Year Old Case. J.Forensic Sciences. Vol. 44: 623-626.
- (4) Walsh, S., et.al. 1989. PCR Inhibition and Bloodstains. Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. Federal Bureau of Investigation. Quantico, Virginia. Pp. 281-282.
- (5) Serology/DNA SOP

15.4 PRACTICE

15.4.1. Safety

Wear lab coat, mask, and gloves when working in the laboratory.

15.4.2. Quality control

Documentation and use of required extraction forms. Reagent blanks for each set of extractions as noted in the DNA SOP. Hair shaft controls for each hair extracted as noted in the DNA SOP.

15.4.3. Reagent preparation

Trainee will prepare any necessary reagents. Trainee may use other extraction reagents already prepared in the laboratory for the DNA section. Any reagents not available in the laboratory will be prepared as necessary by the trainee.

15.4.4. Demonstration by trainer and/or supervised performance

Trainer will demonstrate the steps for separating epithelial cell DNA from sperm cell DNA using one of the extraction methods. Trainer will discuss the remaining extraction procedures in the DNA Technical Manual. Trainer will discuss proper documentation of extracted samples.

15.4.5. Basic skills

Trainee will extract the following using a variety of the extraction techniques:

- 5 bloodstains
- 5 saliva swabs
- 5 hair specimens (optional)

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5 mixed stains (sperm/epithelial cell)

These extractions can be frozen and used for quantitation exercises in Module 16.

15.5 CONCLUSION

Written test

A written test will be administered by the trainer.

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MODULE 16 DNA QUANTITATION

Duration 2 to 5 days

Purpose Enable trainee to quantitate the amount of DNA present in extracted specimens.

16.1 OBJECTIVES

16.1.1. Theoretical

Trainee will be able to:

Understand the necessity for DNA quantitation of extracted samples prior to amplification.

Describe methods for quantitating DNA and the advantages and limitations of each.

Understand and explain the basis for DNA quantitation utilizing the Quantifiler™ kit.

Understand the quality control measures associated with the DNA quantitation method utilized in the laboratory.

16.1.2 Practical

Trainee will be able to:

Prepare dilutions of the human DNA standards as per directions in the Quantifiler™ kit and with Corbett or QIAgility robot.

Perform the quantitation procedure on extracted DNA samples using the Quantifiler™ kit on the 7000/7500.

Calculate the amount of DNA in extracted samples from Quantifiler™ results.

Complete the relevant documentation and quality control for a Quantifiler™ run.

16.2 OUTLINE

() refer to associated references

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- I. Introduction to reading and reference materials
- II. Benefits of DNA quantitation
 - A. DNA testing procedures
 - 1. RFLP requires approximately 50 ng of DNA
 - 2. PCR requires 1-2 ng of DNA
 - B. Preservation of sample for replicate analysis
 - C. Distinguish between inhibition and insufficient DNA quantity
 - D. PCR systems optimized for limited range of DNA quantity
- III. Methods of DNA Quantitation **(1)**
 - A. UV Spectroscopy
 - 1. requires at least 2 ug DNA to detect
 - 2. not primate specific
 - B. Fluorometry
 - 1. requires at least 5 ng of DNA to detect
 - 2. not primate specific
 - 3. requires expensive instrumentation
 - C. Yield Gel
 - 1. requires at least 2 ng of DNA to detect
 - 2. cannot detect single stranded DNA
 - 3. not primate specific
 - D. Quantiblot™ **(2), (3), (6)**
 - 1. sensitivity of 0.02 – 0.15 ng depending on detection method used
 - 2. primate specific
 - 3. can detect single stranded DNA
 - E. Quantifiler™ **(7)**
 - 1. sensitivity of 0.023ng/ul to > 50 ng/ul
 - 2. PCR method
- IV. Quantifiler Protocol **(4), (5)**

16.3 THEORY

Once DNA has been extracted from a reference sample or evidentiary stain, it is important to determine how much DNA is present before continuing with the amplification and typing process. The PCR STR systems utilized in the laboratory are designed to work optimally using a specific range of input DNA. Quantitation of DNA samples prior to amplification can insure that sufficient DNA is added to the PCR reaction and will aid in troubleshooting if no PCR product is obtained. In addition, using minimal volumes of DNA extracts will maximize the number of genetic marker tests or repeat analyses that can be performed.

16.3.1. Limitations

Deleted: Considerations

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It is not possible to associate a positive result with a specific body fluid using this test.

16.3.2. Readings

- (1) Baechtel, F. S. 1989. The extraction, purification and quantification of DNA. Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis, FBI Academy. Quantico, Virginia. Pp. 25-28.
- (2) S. Walsh, et.al. 1992. A Rapid Chemiluminescent Method for Quantitation of Human DNA. Nucleic Acids Research. Vol. 20, No. 19. Pp. 5061-5065.
- (3) Waye, John, et.al. A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts. 1989. Biotechniques Vol. 7 No. 8. Pp. 852-855.
- (4) Austin Police Department Serology/DNA Unit SOP
- (5) User manual for Quantifiler™ Human DNA Quantitation Kit.
- (6) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual. Chapter 4
- (7) Koukoulas, I, et. Al. 2008. Quantifiler Observations of Relevance to Forensic Casework. Journal of Forensic Sciences. 53 (1), 135-141.

16.4 PRACTICE

16.4.1. Safety

Wear lab coat and gloves when working in the laboratory.

Read material safety data sheets (MSDS) for chemicals used in the preparation of the reagents required.

16.4.2. Reagent preparation

None

16.4.3. Quality control

Completion of the required forms, examples of proper documentation, quality control of Quantifiler™ kits, and dilution of human DNA standards.

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16.4.4. Demonstration by trainer and/or supervised performance

Trainee will observe trainer setting up and running a Quantifiler™ run. Trainer will discuss proper documentation on worksheets, interpretation, and calculation of DNA quantities.

16.4.5. Basic skills

Trainee will set up and run a Quantifiler quantitation run™ on previously extracted samples from Module 15. Trainee will complete proper documentation of worksheets, interpretation of results, and calculation of DNA quantities.

16.5 CONCLUSION

Written test

A written test will be administered by the trainer.

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Module 17 AMPLIFICATION

Duration 1 to 2 weeks

Purpose Educate trainee on amplification of human DNA

17.1 OBJECTIVES

17.1.1. Theoretical

Trainee will be able to:

Explain who invented PCR and how the inventor was recognized.

Describe the steps involved in amplification.

Outline the advantages and disadvantages of PCR-based testing.

Deleted: ¶

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

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

Define and describe short tandem repeat (STR) markers.

Define multiplexing and outline the STR markers

Example assessment question: What are the 3 steps involved in amplification? Describe what occurs in each step.

17.1.2. Practical

Trainee will be able to:

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

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

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Complete the amplification worksheet and appropriate documentation for quality control.

Perform the critical reagent quality control check

Use the QIAgility robot for master mix preparation and well spotting

17.2 OUTLINE

() refer to associated references

- I. General information on amplification **(1), (2), (3)**
 - A. Advantages of PCR **(6;pp. 39-51), (7)**
 1. Amount of DNA template required
 2. DNA degradation
 3. Analysis time
 4. Multiplexing
 - B. Disadvantages and considerations of PCR **(7;pp. 113-115)**
 1. Mixed samples
 2. Preferential amplification and stochastic effects
 3. Inhibition
 4. Contamination
 - C. Master mix ingredients
 1. Purpose of each component **(6;pp. 42-43)**
 - a) DNA polymerase
 - b) MgCL₂
 - c) deoxynucleoside triphosphates
 - d) primers
 - e) bovine serum albumin (BSA)
 2. Modification of stringency of amplification by changing concentrations of certain components in master mix **(9)**
 3. QIAgility
 - D. Steps involved in amplification
 1. Purpose of each step **(1;pp. 64-66), (2;pp. 93-94), (6;pp. 39-41), (7;pp. 69-70)**
 - E. Appropriate controls **(4;pg. 5-3), (6;pg.43), (8)**
- II. Background Information on STRs **(6;pp. 53-116), (7;pp. 48-50)**

17.3 THEORY

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

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degraded DNA usually encountered in forensic samples. The use of multiplexed STRs allows for more rapid analysis time with less sample consumption.

17.3.1. Readings

- (1) Sensabaugh, George F. and Cecilia Von Beroldingen. 1991. The Polymerase Chain Reaction: Application to the Analysis of Biological Evidence, *In: Forensic DNA Technology*. Lewis Publishers, Inc. Pp, 63-81.
- (2) Erlich, Henry A., et.al. 1989. The Use of the Polymerase Chain Reaction for Genetic Typing in Forensic Samples *In: Proceedings of a Forensic Science Symposium on the Analysis of DNA*. Pp, 93-100.
- (3) Vosberg, Hans-Peter. 1989. The Polymerase Chain Reaction: An Improved Method for the Analysis of Nucleic Acids. *Human Genetics*. 83: 1-15.
- (4) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual.
- (5) PE Applied Biosystems. AmpF/STR Cofiler PCR Amplification Kit User Bulletin. 1998.
- (6) Butler, John M. 2001. *Forensic DNA Typing*. Academic Press.
- (7) Inman, Keith and Norah Rudin. 1997. *An Introduction to Forensic DNA Analysis*. CRC Press.
- (8) Serology/DNA Technical Manual.
- (9) Moretti, T.R., et.al. 2001. 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. Forensic Sci.* 46(3): 647-660.
- (10) LaFountain, M.J., et.al. 2001. TWGDAM Validation of the AmpFISTR Profiler Plus and AmpFISTR Cofiler STR Multiplex Systems Using Capillary Electrophoresis. *J. Forensic Sci.* 46(5): 1191-1198.

17.4 PRACTICE

17.4.1. Safety

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Wear lab coat, mask, and gloves when working in the laboratory.

17.4.2. Quality control

Completion of required amplification worksheets.

Amplification set-up performed in a dedicated area with dedicated equipment.

Quality control

17.4.3. Demonstration by trainer and/or supervised performance

Trainer will demonstrate the proper technique for setting up the amplification reactions.

17.4.4. Exercises/practice

Trainee will set up amplification reactions (Profiler Plus and Cofiler) from previously extracted and quantified samples along with utilizing the proper quality control worksheets and controls.

17.5 CONCLUSION

Written test

A written test will be administered by the trainer.

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**Module 18 3130 OPERATION, ANALYSIS, INTERPRETATION,
AND TROUBLESHOOTING**

Duration 2-4 weeks

Purpose Educate trainee on the proper use of the ABI Prism 3130 and computer, how to troubleshoot problems with the instrument and make recommendations on fixing any encountered problems, and how to analyze and interpret data from the instrument.

18.1 OBJECTIVES

18.1.1 Theoretical

Trainee will become familiar with the software utilized by the ABI 3130.

Trainee will become familiar with the components used by the computer programs for analysis of data.

Trainee will be able to analyze and interpret data from the ABI 3130

Trainee will learn the proper way to care for and clean the instrument.

Trainee will learn what steps to take in troubleshooting a problem with the instrument.

Trainee will learn how to archive data.

Example assessment question: What is a spectral?

18.1.2. Practical

Trainee will prepare samples manually and with the QIAgility for use on the 3130.

Trainee will perform capillary electrophoresis on previously extracted, quantified, and amplified DNA product.

Trainee will analyze these samples using Genemapper ID and interpret the data.

Trainee will clean the instrument and demonstrate proper care of the instrument.

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Trainee will be given "problem data" and asked to troubleshoot the possible causes and recommend solutions to remedy the problem.

Example assessment question: Observe trainee cleaning and setting up the instrument.

18.2 OUTLINE

() Refers to associated readings

- I. General Information
 - A. Operating system information
 - B. Archiving data and computer maintenance
- II. General information on the ABI Prism 3130 **(4), (5), (6)**
 - A. Multicomponent Analysis **(1), (2;pp. 6-1 to 6-16)**
 - 1. Spectral
 - a) Purpose of the spectral
 - b) Making a spectral
 - 2. Offscale data
 - a) Relative Fluorescence Units (RFU)
 - b) Causes
 - B. Proper care and cleaning of the instrument **(2;pp. 8-1 to 8-20), (4)**
 - 1. Parts of the instrument
 - a) Laser
 - b) CCD
 - c) Mother board
 - d) Autosampler
 - e) Syringe pump
 - f) Heat block
 - g) Pump block
 - 2. Instrument break-down and cleaning
 - a) Reasons
 - b) Frequency
 - c) Pump block
 - d) Syringe
 - e) Water/Buffer vials and septa
 - f) Capillary storage
 - 3. Rebooting the instrument
 - a) Soft reboot
 - b) Hard reboot
 - c) Resetting the instrument
 - d) Re-sending firmware and entering CCD axis values
 - C. Sample preparation/electrophoresis **(1), (2;pp. 8-1 to 8-20), (3), (4)**
 - 1. Instrument set-up
 - a) Capillary alignment

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- b) Filling the syringe and pump block with polymer
 - c) Making buffer
 - 2. Sample set-up
 - a) Formamide/ROX
 - b) Adding sample
 - c) Denaturing and quick freeze
 - d) QIAgility operation
- III. ABI Software, Data Analysis, and Interpretation of data
 - A. ABI Data Collection **(2;1-1 to 1-10 and 8-1 to 8-20), (3), (4)**
 - 1. Laser
 - 2. CCD camera
 - 3. Fluorescent dye labels
 - 4. Manual control of the instrument
 - B. Genemapper ID **(1), (2;8-1 to 8-20 and 9-1 to 9-34), (3),(4)**
 - 1. Analysis parameters
 - a) Setting threshold values
 - b) Smoothing
 - c) Analysis range and excluding primer peak
 - d) Local southern method for sizing
 - 2. Examination of Raw Data
 - 3. Allelic Ladder
 - 4. GS ROX 500
 - a) Defining the internal lane size standard
 - b) Examining the 246 base pair fragment
 - 5. Labeling peaks with base pair sizing, peak height, etc.
 - 6. Extra peaks in the electropherogram
 - a) Off-ladder alleles
 - 1) Spikes
 - 2) Pull-up
 - b) Stutter
 - c) Minus A
 - 7. Microvariants
 - a) Sizing
 - b) CODIS entry when outside the allelic ladder
 - C. Interpretation of analyzed data **(1), (2;9-1 to 9-34 and 10-1 to 10-16),(3)**
 - 1. Controls
 - a) Positive control
 - b) Negative control
 - c) Reagent Blanks
 - d) Allelic Ladder
 - 2. Redundant loci- Amelogenin, D3S1358, and D7S820
 - 3. Inclusions

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4. Exclusions
 5. Mixtures
 - a) Peak height ratios
 - b) Major component
 - c) Minor component
 6. Second reading by qualified reviewer
 7. Stochastic effect and allelic dropout
 - a) Degraded DNA
 - b) Low template number DNA
- IV. Documentation **(1)**
1. Maintenance
 2. Capillary changes
 3. Polymer lot #
 4. ROX lot #
 5. Analysis parameters
 6. Matrix files
- A. Case folder
1. Electropherograms
 2. Controls
- V. Troubleshooting **(1); (2;pp. 11-1 to 11-8 and 9-1 to 9-34)**
- A. Identifying problems with data
- B. Determining causes of problems
1. Urea crystals
 2. Inhibition
 3. Formation of formate ions
 4. Bubbles
 5. Breakdown of buffer vial septa
 6. Old buffer
 7. Bad injections
 8. Bad laser
 9. Bad CCD camera
 10. Dirty capillary window or bad capillary
 11. Arcing
- C. Determining solutions to problems

18.3 THEORY

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.

Data gathered by the instrument must be analyzed and interpreted by qualified analysts. The conclusions 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.

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

18.3.1. Readings

- (1) Austin Police Department Serology/DNA SOP and Technical Manual
- (2) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual. Pp. 1-1 to 1-10; 6-1 to 6-16; 8-1 to 8-20; 9-1 to 9-34; 10-1 to 10-16; and 11-1 to 11-8
- (3) PE Applied Biosystems. AmpF/STR Cofiler PCR Amplification Kit User Bulletin. 1998. Pp. 1-1 to 1-10; 2-1 to 2-23
- (4) Manuals and Inserts for computers, ABI 3130 and associated software.
- (5) Buel, Eric, et.al. 1998. Capillary Electrophoresis STR Analysis: Comparison to Gel-Based Systems. *Journal of Forensic Science*. 43(1): 164-170.
- (6) Fregeau, Chantal J. and Ron M. Fourney. 1993. DNA Typing with Fluorescently Tagged Short Tandem Repeats: A Sensitive and Accurate Approach to Human Identification. *Biotechniques*. 15(1): 100-119.
- (7) Walsh, P. Sean, et.al. 1996. Sequence Analysis and Characterization of Stutter Products at the Tetranucleotide Repeat Locus vWA. *Nucleic Acids Research*. 24(14): 2807-2812.

18.4 PRACTICE

18.4.1. Safety

Wear lab coat and gloves when working in the laboratory.

Read MSDS's for chemicals used in laboratory.

18.4.2 Quality control

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.

Completion of required forms and examples of proper documentation.

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18.4.3. Demonstration by trainer and /or supervised performance

Trainer will demonstrate proper technique for cleaning and setting up the ABI Prism, analyzing and interpreting data, and troubleshooting problems with the instrument.

18.4.4. Exercises/practice

Trainee will analyze previously amplified samples on the ABI Prism and interpret the data. Trainee will troubleshoot problems using the data and suggest/implement ways to remedy these problems.

18.5 CONCLUSION

18.5.1. Written test

A written test will be administered by the trainer.

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MODULE 19 DILUTION AND MIXTURE STUDIES

Duration 1 to 2 weeks

Purpose To provide experience in interpreting mixtures of varying concentrations and in understanding the effects of DNA quantity on typing results.

19.1 OBJECTIVES

19.1.1. Theoretical

Trainee will be able to:

Understand the challenges and limitations involved when working with small/dilute bloodstains.

Gain experience in how DNA quantity of a sample affects typing results.

Know the criteria for determining if a sample is single source or a mixture.

Understand how to apply the 70% rule to determine the major component of a mixed source.

Gain experience in mixture interpretation.

Example assessment question: What are the three criteria for identifying a sample as a mixture? Explain the theory behind each criteria.

19.1.2. Practical

Trainee will be able to:

Prepare and amplify dilution and mixture samples.

Analyze and interpret the dilution and mixture samples using the ABI Prism 3130 and corresponding software.

Examine electropherograms to differentiate between the major and minor components, stutter, and artifacts.

Conclude which individuals would be excluded or included in mixed samples.

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Example assessment question: Perform the 70% rule on mixture samples where appropriate.

19.2 OUTLINE

() – refer to associated references

- I. Dilution Studies
 - A. DNA concentrations requirements **(5)**
 - B. Sensitivity within each 310 instrument **(5)**
 - C. Balance across loci
 - D. Heterozygous peak height balance
- II. Mixture Studies **(1)**
 - A. Major and minor component identification
 - B. Balance across loci
 - C. Heterozygous peak height balance
 - D. Minor peak vs. stutter product vs. artifact
 - E. Diploid vs. haploid cell contributions

19.3 THEORY

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.

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

19.3.1. Readings

- (1) Serology/DNA SOP and Technical Manual.
- (2) PE Applied Biosystems. AmpF/STR Profiler Plus PCR Amplification Kit User's Manual. Chapter 9.
- (3) Duewer, et.al. 2001. NIST Mixed Stain Studies #1 and #2: Interlaboratory Comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples. Journal of Forensic Sciences. 46(5):1199-1210.
- (4) Butler, John M. 2001. Forensic DNA Typing. Academic Press. Pp. 33, 43, 44, 213. Chapters 6, 7, 13.
- (5) Local laboratory's 3130 implementation validation – sensitivity (dilution) study data and mixture study data, if available.

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- (6) Holt, Cydne L., et.al. 2001. TWGDAM Validation of AmpFISTR PCR Amplification Kits for Forensic DNA Casework. Journal of Forensic Sciences. 47(1):66-96.

19.4 PRACTICE

19.4.1. Safety

Wear lab coat and gloves when working in the laboratory. A mask may be worn to further protect against contamination. Safety glasses may be required during some procedures.

19.4.2. Training samples and exercises

I. Dilution Samples

Obtain 3 DNA extracts previously quantitated. Prepare a series of serial dilutions from each extract .

Amplify the samples.

Analyze the samples on the 3130

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

II. Mixture Samples

Read the information on mixture interpretation (2).

Obtain two DNA extracts one from a male (donor A) and one from a female (donor B).

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

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

Amplify the samples

Analyze the samples on the 3130.

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

III. Exclusion and Inclusion Exercise

The trainee will be given 5 to 10 sets of DNA mixture data for the purpose of determining which individuals would be excluded or included.

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

19.4.3. Quality Control

Appropriate documentation and use of approved forms is required.

19.5 CONCLUSION

Written Test

A written test will be administered by the trainer.

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Module 20 STATISTICS

Deleted: . . .

Duration 3 to 5 days

Purpose To familiarize the trainee with the theory of Population Statistics

20.1 OBJECTIVES

20.1.1. Theoretical

The trainee will be able to describe basic principles of population statistics including Hardy-Weinberg equilibrium, Linkage equilibrium, population substructure, the ceiling principle, and random match vs. likelihood ratios.

The trainee will become familiar with the statistics and databases used by the Austin Police Department DNA lab.

20.1.2. Practical

The trainee will be able to manually calculate a single source significance and a mixed source significance estimation using the NRC II Recommendation 4.1 formulae and the Population frequency tables **(1)**.

20.2 OUTLINE

() refer to associated references

- I. Overview of population genetics
 - A. PCR-based systems (2;pp. 21-23, 116-121)
 1. DQ-Alpha
 2. D1S80
 3. STR
 - B. Randomly Mating Populations (2;pp. 26-28)
 1. Hardy-Weinberg (HG) equilibrium (2;pp. 26, 90-98)
 2. Linkage equilibrium (LE) (2;pp. 27, 106-108)
 - C. Population structure (2;pg. 28)
 - D. Subpopulations (2;pp. 28-30,99-106)
 - E. Statistical considerations
 1. The reference database (2;pg. 30)
 2. Match Probability (random match) vs. Likelihood Ratio (LR)
Bayes's Theorem (2;pp. 31-32)
 3. Identification of suspect by database (2;pg. 32)
 4. Uniqueness (2;pp. 33, 161)
 - F. The Ceiling Principles (2;pp. 35, 156-159)
 - G. DNA in the courts (2;pp. 36-42, 50-53)

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- II. The 1992 NRC Report (2;pp. 47-60)
 - A. The validity of DNA typing
 - B. The use of DNA for exclusion
 - C. Changes since the 1992 report
 - D. Paternity testing
- III. Conclusions and Recommendations of the NRC (2;pp.122-124)
- IV. Use of Statistics by the Austin Police Department DNA lab. (1)
 - A. Significance estimation (1)
 - 1. Single source significance calculation
 - 2. Mixed source significance calculation
 - 3. Minimum and Null Allele frequencies
 - 4. Off-ladder alleles
 - 5. Software
 - B. Population databases (1)
 - C. Population frequency tables (1)

20.3 THEORY

Once a match has been identified and an individual included as a possible source of evidentiary material, the significance of that match is estimated to allow investigators, the legal sector, and ultimately a jury of lay persons to place the appropriate emphasis on the conclusion. Significance estimation will be expressed as an inverse probability of inclusion and likelihood ratios will not be calculated. The latest version of the FBI's Popstats software will be configured and installed to calculate single source and mixed source significance estimates. When the estimate for each calculated population group indicates a defined probability of a random match and results are obtained from all thirteen loci, the reporting statement will include an identity of the source statement.

20.3.1. Readings

- (1) Serology/DNA SOP and Technical Manual
- (2) Committee on DNA Technology in Forensic Science: an Update. II. National Research Council (U.S.). Commission on DNA Technology in Forensic Science: an Update. 1996. The Evaluation of Forensic DNA Evidence. National Academy Press. Washington, D.C. Chapter 4, p.89-124, Chapter 5, p.125-162.

20.4 PRACTICE

20.4.1. Training exercises

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The trainee will be able to manually calculate a single source significance and a mixed source significance estimation using the NRC II Recommendation 4.1 formulae and the Population frequency tables **(1)**.

The trainee will be able to apply the *Popstats* software from the FBI.

20.4.2. Supervised performance

The trainer will demonstrate the *Popstats* software.

20.5 CONCLUSION

Written test

The trainer will administer a written test.

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MODULE 21 CODIS CASES

Duration ½ to 1 day

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

21.1 OBJECTIVES

21.1.1. Theoretical

Trainee will be able to discuss and define what a database is, distinguish between different databases, and different indexes (NDIS, LDIS, SDIS).

Trainee will utilize CODIS programs (POPstats) for calculations of statistics.

Trainee will learn the background and state and federal laws associated with the collection of samples for CODIS.

21.1.2. Practical

Trainee will calculate population statistics with POPstats program in CODIS.

Trainee will be able to determine which profiles should be entered into the CODIS database.

21.2 OUTLINE

() refer to associated references

- I. COmbined DNA Index System (CODIS) (1)
 - A. History
 1. DNA Identification Act-1994
 2. House Bill 40, Texas 74th Legislature-Collection of convicted Sex Offender Database.
 3. House Bill 1188, Texas 76th Legislature-Expands collection to murder, aggravated assault, burglary of habitant, offense or conviction of which registration as a sex offender is required.
 4. State Bill 638, Expands the type of sexual offenses for which DNA can be collected
 5. House Bill 656, Statue of limitation on sexual assault cases
 - a) 10 years without DNA analysis

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- b) No limitation with DNA analysis that results in non-victim profile
- B. Indexes **(1)**
 - 1. NDIS-National DNA Index System
 - 2. LDIS-Local DNA Index System
 - 3. SDIS-State DNA Index System
- C. CODIS casework database **(1), (3)**
 - 1. POPstats
 - 2. Specimen Identification
 - 3. Forensic Unknowns
 - 4. Suspect Knowns
 - 5. Forensic Mixtures-resolving mixtures for CODIS entry
 - a) Peak height ratio calculations
 - b) Major/minor component
- D. CODIS reporting **(1)**
- E. State CODIS Laboratory **(1)**
 - 1. Forensic Hits
 - a) Case to case
 - b) Case to suspect
 - 2. Offender Hits
 - 3. Autosearching
 - 4. Uploads
 - 5. Backups

21.3 THEORY

“ The CODIS blends forensic science and computer technology into an effective tool for solving violent crimes. CODIS enables Federal, State and Local crime laboratories to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders.” (2)

The FBI's DNA & Databasing initiatives, US Department of Justice

21.3.1. Readings

- (1) Serology/DNA SOP and Technical Manual.
- (2) The FBI's DNA & Databasing initiatives. US Department of Justice. Oct 2000
- (3) National Research Council. 1998. The Evaluation of Forensic DNA Evidence. National Academy Press.

21.4 PRACTICE

None.

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21.5 CONCLUSION

Written test
None.

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TRAINING MANUAL

Module 22 REPORT WRITING AND CASE REVIEW II

Duration 1 to 2 weeks

Purpose To provide guidelines for DNA casework report writing and for technical reviews and administrative case reviews.

22.1 OBJECTIVES

22.1.1. Theoretical

Trainee will be able to write clear, concise reports that contain DNA analysis.

Trainee will be able to perform technical case reviews for DNA casework.

Example assessment question: On what laboratory form is the technical review documented?

22.1.2. Practical

Trainee will be able to compile DNA results into a concise conclusion.

Trainee will be able to technically review a DNA case from the testing performed to the written report.

Trainee will be able to produce a report in an easily understandable format.

Example assessment question: How does the technical review differ from the administrative review?

22.2. OUTLINE

() refer to associated references

- I. Report writing
 - A. General Information – laboratory reports **(1; 6)**
 - B. Evidence submitted - if the letter containing DNA analysis is a supplemental letter, then only the items analyzed for DNA need to be listed. If this is the original letter on the case, then all items submitted should be listed.
 - C. Requested analysis - the requested analysis can be a mixture of the laboratory's normally used statements and any special requests made by the officer.
 - D. Results of DNA analysis **(2)**

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- F. Statistics **(2)**
- G. Disposition
- H. Counting Sheet
- II. Case review
 - A. Technical Review **(1), (3;standard 12.1)**
 - B. Documentation - the technical review may be performed on a rough draft or on the final report. The case information should be organized in a logical fashion with case items highlighted on relevant QC forms. The DNA paperwork should be arranged in chronological order.
 - C. Administrative Review **(1;5.3), (3;standard 12.1)**

22.3 THEORY

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

22.3.1. Readings

- (1) Forensic Science Division SOP
- (2) Serology/DNA SOP and Technical Manual
- (3) FBI, "Quality Assurance Standards for Forensic DNA Testing Laboratories" Standard 12.1

22.4 PRACTICE

22.4.1. Quality control

I. Report writing

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. All results or conclusions in the formal report must be 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.

II. Technical review

The review is an evaluation of documentation to check for consistency, accuracy, and completeness. The review is an evaluation of reports, notes, data and other documents to ensure an appropriate and sufficient basis for the scientific conclusions.

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The technical review must be conducted by a second qualified analyst.

22.4.2. Practice

1. The trainer will provide 10-20 cases involving DNA analysis to the trainee for review to allow familiarity with the report writing format in the laboratory.
2. The trainee will be provided with data from 20 cases previously analyzed and reported.
3. Using the Serology/DNA SOP and instruction from the trainer, the trainee must interpret the results and prepare a report

22.5 CONCLUSION

Written test

None.

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Module 23 TECHNICIAN TRAINING

Duration 1 week

Purpose To provide guidelines for technicians in the DNA section

23.1 OBJECTIVES

23.1.1. Theoretical

Technician will become familiar with City, APD and laboratory policies

Technician will become familiar with laboratory equipment and reagents

23.1.2. Practical

Technician will be able to perform general maintenance on equipment

Technician will be able to prepare reagents

23.2 OUTLINE

() refer to associated references

- I. Read Manuals
 - A. General Orders
 Forensic Division Manual
 DNA SOP
 Safety Manual
- II. Attend Safety Training
- III. Reagent Preparation
- III. Equipment Maintenance
- IV. Equipment QC
- V. Become familiar with the LIMS system

23.3 THEORY

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The technician will provide support to the DNA section as needed. If the technician performs casework duties then the technician will be trained in the relevant modules and will take a competence test on those tasks.

23.3.1. Readings

- (1) Forensic Science Division SOP
- (2) Serology/DNA SOP and Technical Manual
- (3) APD Policy Manual
- (4) Safety Manual

23.4 PRACTICE

23.4.1. Practice

1. The technician will make reagents under the direction of an analyst.
2. The technician will maintain equipment under the direction of an analyst
3. The technician will QC equipment and reagents under the direction of an analyst

23.5 CONCLUSION

Written test

None unless technician performing casework. Then relevant exams will be given based on tasks performed.

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