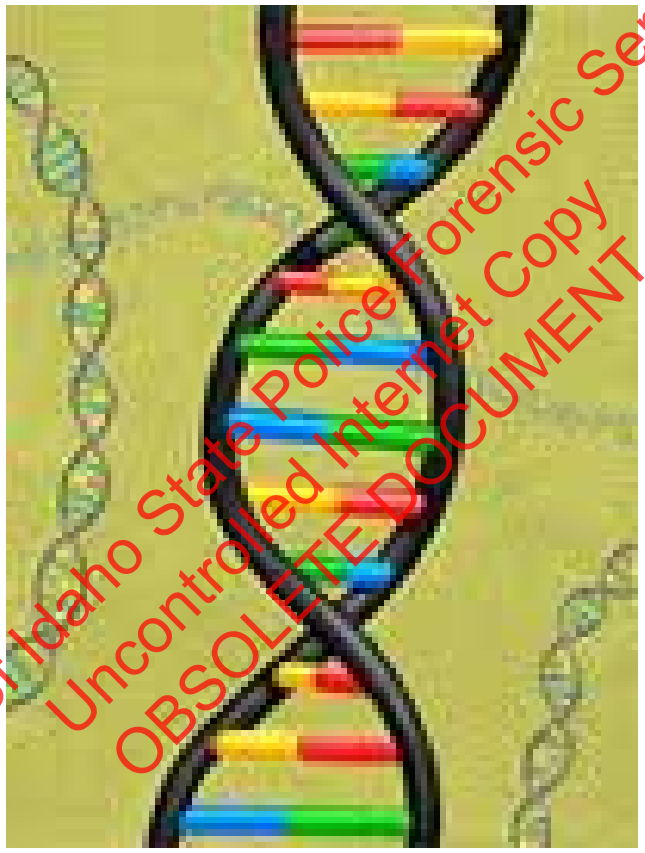
FORENSIC BIOLOGY TRAINING MANUAL



Revision 2
5/23/12
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Forensic Biology Training Manual

Revision #2

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REVISION RECORD

The following table must be filled out when revisions to the Forensic Biology Training Manual are made.

Date: The effective date of the revision(s).
Revision #: The manual revision number.
Description: A brief description of the changes made to the manual.
Addition: This column is checked if the revision reflects an addition (e.g. new SOP or form) to the manual.
Deletion: This column is checked if the revision reflects a deletion (e.g. SOP or form no longer in use) from the manual.
Initials: Initials of the Technical Leader making the revisions.

Date	Revision #	Description	Addition	Deletion	Initials
9/6/11	1	Rewrite of all sections, reformatting, expanded exercises and evaluation form, added review and CODIS modules, added database/casework specific background and exercises	X		CRC
5/23/12	2	Revise DNA Database to reflect automation, added BBP and Chemical Hygeine training to module 1, replaced 9947A with 2800M	X		CRC

Table of Contents

INTRODUCTION

The purpose of this manual is to provide an in-house training program designed to prepare the trainee with the theoretical and practical background necessary to perform reliable analysis. The program is composed of a series of modules consisting of reading materials, observation and demonstration, practical exercises, and demonstration of competency. A competency test may consist of a written, oral, or hands-on practical test, or any combination of these. Whenever possible or practical, the training modules may be supplemented with technical lectures provided by qualified analysts. Progress of the trainee will be monitored through the use of a training evaluation form (refer to **Form 100-TR**), in which successful completion of a module is indicated by the signatures of the trainee, trainer, and/or technical leader. External training, where available, may also be used to supplement and/or meet certain portions of the training program, as appropriate.

The training program, in its entirety, is designed for the trainee who has no prior background or experience in the subject matter. The modules outlined are the minimum requirements for completion of training; however, additional exercises may be assigned, at the discretion of the technical leader, if necessary. Alternatively, the training may be abbreviated for analysts with experience and training from another organization or those individuals who perform only limited duties. The background and experience of the individual will be assessed prior to beginning the training program. This assessment will be made by the individual's technical leader. In some instances, it may be sufficient for the experienced analyst to simply complete a competency test prior to beginning analysis.

The training modules do not need to be completed in sequence. Depending on the needs of the laboratory and the trainee's experience level, certain modules may be prioritized and at their completion the trainee may be allowed to perform limited work in that area.

Roles and Responsibilities

Technical Leader	The unit technical leader is responsible for monitoring the training process and for the final approval of the trainee's release to process casework or offender samples. The technical leader should regularly monitor the trainee's progress and review their training binder for completeness and accuracy. At a minimum, the technical leader should meet with the trainee at the end of each module to discuss the exercises and any further actions.

Trainer

The trainer will be the unit technical leader or their designee. He/she is responsible for demonstrating a particular technique and observing the trainee perform the same procedure where applicable. This process should include the following elements: demonstration/instruction by the trainer; technique performed by the trainee/instruction given by the trainer; technique performed by the trainer/instruction given by the trainee; demonstration/instruction by the trainee. Information gained from reading materials should be reinforced through detailed discussion of the technique during the demonstration and/or observation. This information should include both theoretical and practical aspects.

The trainer is also responsible for verifying that the trainee is meeting each of the milestones leading to the successful completion of the module and/or training program. The trainer's initials on the training evaluation form and training logs indicate that the trainee has successfully completed the observation or exercise on the date indicated. Exercises and competency tests will be evaluated by the trainer and/or the unit technical leader prior to initialing and dating the checklist. In addition, Supervised Casework and Technical Review logs will be completed by the trainer. The trainer, if not the technical leader, shall periodically meet with the technical leader to discuss the progress of the trainee.

Trainee

The trainee is responsible for maintaining a training binder or notebook which will contain the records (i.e. notes, worksheets, photographs, etc.) generated during the training program. The trainee's initials on the training evaluation form indicate that the trainee has successfully completed the reading/review of the selected material on the date indicated. The trainee should keep the trainer and unit technical leader informed as to their progress and/or any problems or questions that may arise. The trainee has the ultimate responsibility for learning the materials necessary to successfully complete competency tests and should take an active role in obtaining the information needed (reading, observation, discussing/asking questions, etc.) to do so. The trainee is also responsible for recording each required observation on the appropriate log. At any time a trainee feels that their training is not progressing or that they are experiencing difficulty with the exercises they should meet with their unit technical leader to discuss their situation.

1.0 Module 1: Laboratory Introduction

1.1 Background Information and Theory

Within the Idaho State Police (ISP) there are three Forensic Services laboratories, located in Meridian, Pocatello, and Coeur d'Alene. The function of the laboratories is to provide fair and impartial scientific analysis to the criminal justice system. Although not all services are available at every laboratory, examinations, assistance, and testimony are offered in the following areas: controlled substances, shoe prints, tire tracks, latent prints, forensic biology (screening and DNA), DNA database, crime scenes, clandestine laboratories, toxicology, blood/breath alcohol, firearms, and toolmark analysis. In addition, ISP Forensic Services provides training to law enforcement agencies, attorneys, and medical personnel.

Since 1987, all three laboratories have been accredited by the American Society of Crime Laboratory Directors – Laboratory Accreditation Board (ASCLD-LAB). Laboratory accreditation is granted to those that meet required quality assurance (QA) standards and it helps to provide confidence in the quality of work generated by a lab. QA covers the overall laboratory system and includes measures taken by the lab to monitor, verify, and document performance to demonstrate that a product or service meets specified requirements for quality. Quality control (QC) includes activities done to ensure that a product or service meets specified quality standards. QC is a functional check on QA. In addition to equipment and methods, quality standards also apply to lab staff. Those working in the lab must take a scientific and objective approach to their work, which means being unbiased, using control and reference samples, and keeping accurate records. Both organizations and individuals continue to be challenged to reach higher quality standards.

Universal precautions and safe laboratory technique should be practiced when working with items that potentially contain biological material. Biological materials may contain several infectious agents, which vary in their survival time outside the body. In general, the analyst should treat all items of evidence and offender samples as if they were positive for one or more of these pathogens and take the appropriate precautions (e.g. gloves, laboratory coat, face protection, etc.) when examining them. Proper

decontamination procedures and disposal of contaminated waste should be maintained in order to aid in the individual's protection and the removal of the pathogen.

1.2 Objectives

To gain familiarity with Idaho State Police as an organization, the Forensic Services laboratory, the Biology Section, and the policies and procedures which guide the laboratory operations.

1.3 Practical Exercises

Reading and observation, as outlined in the corresponding evaluation form.

1.4 Principles and Knowledge

The analyst should have an understanding of ISP and the Forensic Services laboratory, which may include but is not limited to the following:

- Orientation to Laboratory/ISP Facilities
- Organizational Structure, Chain of Command, and Policies and Procedures
- Security and Confidentiality Issues (e.g., access codes, visitors, communications, and data privacy)
- Quality Assurance/Quality Control
- Health and Safety Guidelines (e.g., universal precautions, bloodborne pathogens, chemical hygeine, biohazard disposal, decontamination, and vaccinations)
  Note: Safety hazards associated with specific analytical methods and/or instruments will be covered as part of the associated module.
- Location and Use of Safety Equipment
- Introduction to Other Forensic Disciplines
- Ethics in Forensic Science

2.0 Module 2: Evidence/Offender Sample Handling

2.1 Background Information and Theory

2.1.1 Evidence Items

Maintaining evidence integrity is imperative for every forensic science discipline. Several steps should be taken to accomplish this goal. The chain of custody must be maintained from the time of collection until presentation in the courtroom, meaning everyone who handles the evidence must sign for it and record what they did with it. Secure packaging is essential to restrict access to the item and it must not be left unattended to prevent tampering or theft. Evidence must be properly stored, which may involve refrigeration and protection from moisture, to prevent deterioration. Documented procedures must also be followed to minimize loss, contamination and/or deleterious change. It is also necessary to understand other evidentiary requests and needs. All of the examinations, analyses conducted, and samples collected must be properly documented.

All evidence submitted to the laboratory is entered into an evidence tracking or laboratory information system. The system is a computer program used to assign unique laboratory case and item numbers for all submitted evidence. Each item has a corresponding barcode placed on the outer packaging as an identifier, which allows tracking of its movement while in the lab. The system also allows for the maintenance of case information, including items submitted, analyses requested, reports, and status.

2.1.2 Offender Samples

In 1996 Idaho passed a law (§19-5501 – §19-5518) which authorized the creation of a database for the retention of DNA profiles of offenders convicted of specific crimes. ISP Forensic Services began collecting those samples in 2000.

The offender samples are collected, normally by corrections officers or law enforcement personnel, as a buccal swab transferred to FTA paper. The sample is stable for several years at room temperature because the paper

contains chemical substances which protect DNA from degradation by enzymes and bacterial growth.

Offender samples received into the laboratory for entry into the DNA database are not treated as evidence. The received date is documented on each sample and the corresponding information is entered into the computer system (Tracker). Tracker is used to manage offender samples and court orders, including but not limited to the following: identification of duplicates, generation of unique identification numbers, storage of offender details (identifying information and offense), thumbprint/state ID verification for flagging of criminal histories, and tracking the progress of sample processing/testing. To prevent tampering or theft the samples must not be left unattended or unsecured.

2.2 Objectives

To introduce the trainee to the proper procedures for evidence or offender sample acceptance, receipt, documentation, and handling, as well as to become familiar with the computer system used for either Casework or Database sample tracking.

2.3 Practical Exercises

Reading and observation, as outlined in the corresponding evaluation form.

2.4 Principles and Knowledge

The analyst should have an understanding of evidence or offender sample handling, which may include but is not limited to the following:

- Case/Evidence/Offender Sample Acceptance and Receipt
- Computer System (e.g., Evidence Tracking/LIMS, Tracker)
- Chain of Custody Maintenance/Documentation
- Evidence/Offender Sample Packaging and Storage
- Evidence Handling, Retention/Consumption, and Documentation
- Case Assignment (backlog and tracking procedures)

3.0 Module 3: Supplies, Quality Control, and Reagents

3.1 Background Information and Theory

An important part of QA is routine quality control checks such as temperature monitoring, decontamination, and safety equipment checks. It is imperative that the analyst be able to recognize problems, troubleshoot if necessary, and notify the appropriate personnel. Documentation is kept to track the QC checks, decontamination, and any problems observed.

Chemicals and reagents in the laboratory must be properly labeled and include a National Fire Protection Association (NFPA) chemical hazard label. This is a color coded, numerical system to indicate the health, flammability, and reactivity hazard of chemicals; symbols for special precautions may also be included. These requirements also apply to reagents prepared in the laboratory. In addition, documentation of all reagents made in-house and chemicals or reagents received into the lab must be recorded in the appropriate logs.

When working with chemicals, protective equipment should be worn and safe handling methods followed. The material safety data sheet (MSDS) can be a helpful resource for chemical handling and should be available for each substance used in the laboratory. A MSDS contains data regarding the properties of a particular substance. It is intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner. The form includes information such as physical data, toxicity, health effects, reactivity, storage, disposal, protective equipment and procedures for handling spills.

3.2 Objectives

To gain familiarity with ordering of supplies, reagents, and quality control in the Biology Section. After completion of this module the employee should be able to make reagents, order supplies/chemicals, and perform/document routine quality control checks.

3.3 Practical Exercises

Reading, observation of individual procedures (documented on the observation log), and the hands-on exercises listed

below. All reading and observations must be completed prior to performing the corresponding exercises.

- Perform weekly quality control checks.
- Perform non-instrumental monthly quality control checks.
- Perform non-instrumental quarterly quality control checks.

3.4 Principles and Knowledge

The analyst should have an understanding of the laboratory procedures concerning general quality control and reagents/supplies used in the laboratory, which may include but is not limited to the following:

- Reagent Preparation and Log
- Purchasing Card Training
- Ordering and Documentation
- Chemical Inventory
- MSDS and Safe Handling/Use of Chemicals/Reagents
- Labeling and Documentation for Supplies and Chemicals/Reagents Made or Received in the Biology Section
- Quality Control Checks

4.0 Module 4: Biological Screening

4.1 Background Information and Theory

4.1.1 Blood Tests

A suspected bloodstain is first processed using a presumptive test. The test depends upon the catalytic peroxidase-like activity of the heme group of hemoglobin, which catalyzes the oxidation by peroxide of an organic compound (phenolphthalein or o-tolidine) to yield a colored product. These tests are not specific to blood, therefore, a positive color test alone should be interpreted as an indication of blood rather than a positive identification. However, a negative result is indicative of the absence of detectable quantities of blood. Color development before the addition of hydrogen peroxide may be due to the presence of a chemical oxidant. Several other substances may give a positive color reaction for these presumptive tests; however, they can generally be visually distinguished from blood.

The presumptive test is not specific for human blood so once a positive result is obtained, further steps must be taken if confirmation of human origin is necessary. One test is based on the immunological detection of human hemoglobin and the reaction time is dependent on the concentration. Any human hemoglobin present will combine with a dye-labeled antibody to form an antigen-antibody complex. This complex migrates to the test region where it is immobilized and a visible line forms. For positive results, it should be stated that human blood is indicated, rather than detected or identified, as some non-human species, such as higher order primates and members of the mustilidae family, may produce a positive result.

4.1.2 Semen Tests

The acid phosphatase test is used as a presumptive screening test for semen. Naphthyl phosphate is acted upon by the acid phosphatase enzyme to produce naphthol, which then combines with diazo blue B dye to give a violet-colored complex. Seminal acid phosphatase, which originates in the prostate gland of males, occurs at concentrations 20 to 400 times higher than that of other

body fluids and is present at higher concentrations in humans compared to other animal species. Since acid phosphatase occurs in other human tissues, animals, and plants, a positive test does not confirm the presence of semen. On the other hand, since this assay is dependent upon the amount of enzyme present, a negative acid phosphatase test does not necessarily mean that a stain does not contain semen.

The microscopic identification of spermatozoa is a conclusive test to identify the presence of semen. Human spermatozoa have a distinct size and morphology, but sperm cells of some species, other cells, and extraneous objects may appear similar. The "Christmas Tree" staining method is used to increase the contrast of the sperm to aid in identification. The method uses two 'dyes' containing nuclear fast red and picroindigo carmine, which results in nuclear material staining red and other cellular components green. Additionally, sperm exhibit a differential staining pattern in which the acrosomal cap stains a lighter red (pink) than the remainder of the head.

The presence of semen in sperm negative samples is confirmed with the detection of the human semen-specific protein p30. P30 is a glycoprotein produced in the prostate gland and found only in males. The test is based on the immunological detection of p30 and operates in a manner like the confirmatory test for blood. P30 may also be detected in peripheral blood of males with prostate cancer and can be found in urine, probably due to leakage from prostate into urethra. P30 is present in semen of several Old World monkeys, but has not been detected in semen from other mammals.

4.1.3 Saliva Test

Saliva is difficult to conclusively identify because of the lack of sufficient amounts of detectable substances specifically unique to saliva. While it is not unique to saliva, the enzyme α-amylase provides an important parameter for saliva stain identification. Other body fluids that may contain α-amylase include the following: pancreatic juice, fecal material, urine, blood, and semen. Methods for testing α-amylase depend on the enzyme's

ability to hydrolyze starch. Tests to distinguish between salivary and pancreatic amylase exist but are not commonly used in the field today.

4.1.4 Urine Tests

Like saliva, urine is difficult to conclusively identify because of the lack of sufficient amounts of substances unique to urine. Urea and Creatinine are two substances found in high concentrations in urine. These substances can also be found, at lower concentrations, in other fluids including: blood, perspiration, saliva and semen.

Urea is detected using the enzyme urease. Urease causes the urea to be broken down into ammonia and carbon dioxide. The ammonia is then detected using an indicator chemical. It can also be detected using litmus paper as the reaction will cause an increase in pH.

Creatinine is a byproduct of normal metabolism. It can be detected by applying a saturated solution of picric acid. The result is creatinine picrate which will change the color of the solution from yellow to orange.

DNA analysis is typically not performed on urine stains. Urine is composed primarily of water and salts and has a very low cellular concentration. Additionally, the bacterial content in urine can cause DNA degradation of the limited amount of cellular material present.

4.1.5 Feces Test

Fecal matter testing involves the identification of urobilinogen, a byproduct of bilirubin metabolism. Bilirubin is a byproduct of the breakdown of heme, a component of red blood cells. While not unique to fecal matter, it is found in high concentrations in the feces of carnivores and omnivores but will not be found in that of herbivores. Urobilinogen is oxidized to water soluble urobilin, when combined with alcoholic zinc acetate. This compound will fluoresce a bright green color when exposed to UV light.

DNA analysis is typically not performed on fecal matter or stains. Bacteria comprise approximately one third of fecal matter causing rapid DNA degradation. In addition, many inhibitors of PCR are present in feces including:

bile pigments and salts, polysaccharides and bilirubin. If these inhibitors are not removed, traditional extraction methods typically fail to yield a DNA profile from the sample.

4.1.6 Alternate Light Source

Dried stains of physiological fluids such as semen, saliva and urine can be difficult to locate on an item of evidence under normal room lighting conditions. Light travels through an energy spectrum from ultraviolet to gamma radiation. The visible light spectrum is between 400 and 600 nanometers (nm). Under standard lighting conditions (normal room lighting) dry semen stains are visible at approximately 350 to 400nm, which is below the range of visibility for the unaided eye. If the stain is exposed to light of a longer wavelength/lower energy (ultraviolet (UV) light), in a darkened room, particle excitation occurs causing fluorescence. Under these conditions semen stains have a strong visible fluorescence at approximately 450 nm. In order to visualize this fluorescence a filter must be used to block all light except for that within the range of 450nm. This is done by using orange colored goggles. Other substances may also fluoresce at these wavelengths of light which is why follow-up with a presumptive chemical test is necessary.

4.1.7 Infra-Red (IR)

Bloodstains on dark colored fabrics such as black fabrics and denims can be difficult to visualize. IR lighting/photography can be used to aid the analyst in locating these stains. Dark fabrics and bloodstains will absorb most of the light in the visible spectrum, making it difficult to distinguish the stain from the background. Infrared lighting (700 to 900nm) causes dark fabrics to reflect visible and infrared wavelengths of light. If present, a bloodstain on the fabric will typically still absorb both wavelengths of light. This will result in the background/fabric appearing white or grey while the bloodstain will still appear dark in color.

4.1.8 Evidence Examination/Sample Selection

Once the methods for locating and identifying potential biological evidence have been learned, it is important to

be able to put them to use in examining evidentiary materials. During evidence examination, one should be aware of the potential importance of other types of analyses and every effort should be made to document, conserve, and/or collect these samples (e.g., bloodstain patterns, trace evidence, latent fingerprints, and DNA) so they are not lost. The presence of other types of evidence is useful in directing the examinations conducted and the order in which to do them. It is important to evaluate the significance of biological stains in relation to the evidence item and the entire case, especially in determining stains/samples to be selected for analysis. In order to accomplish this, a police report should be obtained to assist in directing/focusing the evaluation and analysis. When sample selection occurs, the report shall clearly indicate what was tested and make no inference about the entire population.

The following measures must be taken to avoid contamination of evidence during the examination process: examine victim and suspect evidence and/or evidence and reference samples separately, examine one item of evidence at a time, use clean examination paper for each item, properly clean implements and workbench, and change gloves as necessary. This is especially important with the increased sensitivity of Polymerase Chain Reaction (PCR) DNA typing methods.

4.2 Objectives

Gain the knowledge base and mastery of the technical skills necessary to examine/process items of evidence for the presence of biological substances. This includes, but is not limited to the following: safe handling of biohazards; observation, documentation, sample selection, and collection of stains (and trace material where appropriate); body fluid identification; and communication of findings through written reports. Upon successful completion of this module, an analyst will be able to perform casework processing up to and including the assembly of a "DNA Packet", write evidence screening reports, conduct QC checks of critical screening reagents, and participate in research and/or validation relevant to the development and improvement of screening techniques employed in the Biology Section.

4.3 Practical Exercises

Reading, observation of individual procedures (documented on the observation log), and the hands-on exercises listed below. All reading and observations must be completed prior to performing the corresponding exercises.

Note: Samples that are collected and/or prepared for use in the hands-on exercises may be retained for use in future testing exercises. Some samples may include those previously collected and stored frozen.

4.3.1 Blood/Semen Sensitivity

- Prepare serial dilutions of liquid blood and semen to at least ≈1/10,000. Place 50 µl of each dilution onto clean white cloth and allow to air dry.
- Test the blood dilutions with the available presumptive reagents using the filter paper scratch method. At the point a negative result is obtained, begin taking minimally sized cuttings and test them directly until a negative result is achieved.
- Test a variety of 'aged' bloodstains with each presumptive reagent (use direct cuttings only as needed).
- Test the semen dilutions with freshly prepared acid phosphatase reagent using the moistened swab method. At the point a negative result is obtained, begin taking minimally sized cuttings and test them directly until a negative result is achieved. Additionally, choose a high concentration and a low concentration stain and perform AP mapping on them.
- Test a variety of 'aged' semen stains with the acid phosphatase reagent, as well as semen-stained vaginal swabs at varying post-coital intervals up to at least 48 hours and from at least two different couples (one set is to be freshly collected and, if available, the other previously collected and stored frozen).

4.3.2 Blood/Semen Specificity:

- Prepare stains from a wide variety of body fluids, fruits, vegetables, and other substances. Test the stains using each of the presumptive blood and acid phosphatase reagents. It may be desirable to test some items (e.g. fruits and vegetables) fresh as well (consult the literature and trainer for specific items to include).
- Test various non-human bloodstains (as available) using the presumptive blood reagents.

4.3.3 Alternate Light Source

- View all stains prepared thus far (blood and semen dilutions, body fluids, fruits, and vegetables) with the alternate light source (at each available wavelength) and note any observations (under both room and UV light).
- Place a small amount of semen on a variety of unstained fabrics/substrates (to include, but not limited to black fabric, denim, and floral-type patterns). View each with the alternate light source at each available wavelength for the observation of any inherent fluorescence, quenching, repetitive patterns and/or any effect from the substrate.

4.3.4 IR Cameras/Stereomicroscope

- Place a variety of bloodstains (to include fine spatter, drips, and transfer stains) onto a minimum of two different dark colored fabrics (one of which is black). Allow the stains to air-dry and view them under bright light using the stereomicroscope, taking notes on overall appearance of the stains. If available, also examine the previously stained black panties.
- Examine the above items with the available IR cameras. Note observations regarding color, general stain appearance, and ease of visualization under IR vs. room lighting. Photograph a representative sample of the stains under both lighting conditions.

4.3.5 Microscopic Exam for Sperm

- View the photos of various animal sperm and describe (may include a sketch) the general appearance and stain patterns of each. Various yeast photos should also be reviewed as a general guideline only.
- Prepare an unstained slide and a 'Christmas Tree' stained slide of neat semen. Examine both slides under the microscope (using various powers of magnification) and note the general appearance of sperm and/or any other cells observed on each.
- Extract each of the previously prepared semen dilution stains and prepare a stained slide. View each of these slides under the microscope and note any observations. Include the approximate number of spermatozoa observed for each using both descriptive (i.e. few, moderate, many, etc.) and/or numeric (i.e. #/200X and 1+ - 4+) terms. Additionally, note any other cells observed. Document one slide representing each of the numeric ratings with a photograph. The Trainer will review a representative sample of these slides to confirm the rating of the number of sperm present.
- Prepare stained slides of semen-free vaginal swabs, buccal swabs, an unused lubricated condom swab, a neat aspermic semen stain, a bloodstain and the previously examined post-coital swabs. View these under the microscope and note the general appearance, numbers, and stain patterns of any cells observed.

  Note: All of the remaining extracts from above should be retained for use in p30 exercises.

4.3.6 P30

Prepare or use previously prepared extracts for the following samples: semen dilution stains, post-coital swabs, aged semen stains, and body fluids (must include a urine sample collected from a male). Test each of the extracts using the Abacus P30 test cards, according to the laboratory protocol.

4.3.7 Hematrace

Test the following items according to laboratory protocol, using the Abacus Hematrace test cards: previously prepared bloodstain dilution series, various aged bloodstains, body fluids, and animal bloodstains (as available).

4.3.8 Saliva

- Obtain a fresh saliva sample and prepare a dilution series to 1:1,000. Using the analytical method for Phadebas, test each of these dilutions to determine the sensitivity of the method. Additionally, saliva samples deposited on at least five different substrates (e.g. gum, eating utensils, etc.) and known saliva (neat, 1:100, and 1:500) from at least two different individuals are to be tested according to laboratory protocol.
- The specificity of the test method should also be determined by testing various body fluids and animal saliva, as available.

4.3.9 Urine

- Obtain a fresh urine sample and prepare a dilution series to 1:500. Using the analytical methods for Urease and Creatinine (as available), test each of these dilutions to determine the sensitivity of the method.
- The specificity of the test method should also be determined by testing various body fluids and animal urine, as available.

4.3.10 Feces

The following samples are to be tested using the analytical method for Urobilinogen: fresh human fecal stain, various body fluids, fecal stains from various animals as available, and a stain prepared from Vegemite. Slides made from extracts of the human fecal and Vegemite stains are also to be prepared, stained, and viewed under the microscope for

observation of general appearance and any cells present.

4.3.11 Case Review and Hands of the Analyst

- Review a minimum of ten case files (encompassing multiple sample types and analysts) to include note format, evidence description, sketches/photographs, sample selection, report writing, and communication logs.
- Process casework samples while under constant observation by the trainer or designated qualified analyst in custody of the items. The trainee will handle, examine and perform testing on each item. The case analyst will provide case documentation, with the trainee initialing test results. The report will be issued by the qualified analyst/trainer; however, the trainee will also prepare a summary of the testing results using the approved results statements listed in the biology/DNA quality manual.

4.3.12 Mock Cases

Process a minimum of two mock biological screening cases and write reports for each. The documentation and analysis of items are to be performed as would be done in the course of normal casework.

Note: non-probative cases may be processed in addition to mock cases as available.

4.3.13 Quality Control

Perform critical reagents kit QC.

4.4 Principles/Knowledge:

The analyst should have an understanding of biological evidence screening, which may include but is not limited to the following:

- Evidence Acceptance Policies and Testing Schemes

- Understand Screening Tests (e.g., theory, mechanism, sensitivity, limitations, various techniques, utility of controls, and safety hazards)
- Application of Biology Protocols for the Identification of Body Fluids
- Methods of Evidence Documentation
- Methods to Avoid Contamination
- Sample Selection Methods and Conclusions
- Case File Content and Organization
- Report Writing and Communication
- Quality Control

5.0 Module 5: DNA Analysis

5.1 Background Information and Theory

DNA (Deoxyribonucleic Acid) is genetic material found in the nucleus of most cells in the body. DNA is comprised of nucleotide units that are made up of three parts: a nucleobase, a sugar, and a phosphate. Half of a person's DNA information comes from their mother and the other half from their father. The variation passed down in the form of alleles, is what makes DNA analysis such a powerful tool in the field of forensics (Butler, 2001).

A wide range of equipment is used in the process of conducting DNA analyses. It is important to ensure that the staff is trained to properly use the equipment and that it is regularly maintained and/or calibrated. Documentation is kept to track periodic function checks, maintenance, problems, and repairs or replacement of equipment.

5.1.1 History

DNA analysis has been used in the field of Forensics since 1985. Prior to DNA typing, conventional serological methods were utilized; however, they did not allow the analyst to make conclusive statements as to the identity of an individual. The first type of forensic DNA analysis used was restriction fragment length polymorphism (RFLP). RFLP is powerful in its ability to differentiate individuals; however, its application to forensic science is limited because of the large sample size required. PCR (polymerase chain reaction) was introduced into the field of forensic biology to address these limitations.

PCR has been successful in yielding genetic information from degraded samples and those of limited quantity. Early commercial kits (AmpliType HLA DQ-∝, AmpliType PolyMarker and D1S80) were successful at typing degraded samples, but their discriminating power was lower than the RFLP method. This and other limitations lead to the development of the current method used in forensic DNA analysis, short tandem repeats (STRs). STRs are stretches of highly polymorphic, repetitive DNA sequences. The STR method has the benefits of the other PCR based systems but without most of the limiting factors, such as lack of

discrimination and clearly defined statistics. PCR based systems are highly sensitive, therefore special handling techniques must be used to prevent sample contamination.

5.1.2 Extraction

DNA must first be isolated from other cellular components and any non-biological material, as any residual material may inhibit subsequent analysis. The extraction procedure varies somewhat according to the type of biological evidence present, the amount of evidence, the kinds of cells present, and downstream processes.

Chelex extraction is a rapid method in which the samples are boiled in a solution containing beads of a chemical called Chelex. Boiling breaks open the cells to release the DNA and the Chelex binds extraneous materials, which might interfere in the analysis. The result is isolation of single-stranded DNA.

Organic extraction involves the serial addition of several chemicals to lyse cells and organic solvents to separate the high molecular weight, double-stranded DNA from cellular components. The DNA is further purified and concentrated using special filters.

Silica coated magnetic particles can be used to rapidly purify DNA. DNA in sample lysates binds to the silica surface of the particles in the presence of a chaotropic salt. The particles are then separated using a magnetic source, while other components are washed away. DNA can then be eluted in water or a low-salt buffer. This method can be used to clean-up inhibitors following other methods and is ideal for automated liquid handling with robotics.

Differential extraction relies on the different properties of epithelial cells and sperm cells in order to separate them from each other before DNA is isolated. This procedure involves preferentially breaking open the epithelial cells with chemicals and then isolating them from the sperm cell fraction. The sperm cells are then lysed with a different set of chemicals. Both fractions are then subjected to the organic or magnetic particle extraction.

FTA paper causes cells to lyse upon contact with the paper so a portion of the paper need only be washed with extraction buffer to remove inhibitors and purify the DNA.

5.1.3 Quantitation

Before the analysis proceeds, it is imperative to determine the quantity and quality of DNA. Because a narrow concentration range is optimal, determination of the amount and condition of DNA in a sample is essential for most PCR-based systems. Too much input DNA can result in peaks that are off-scale for the measurement technique. Too little template DNA may result in allele 'drop-out' because the PCR reaction fails to amplify the DNA properly.

Real-time PCR (RT-PCR) or quantitative RT-PCR is a technique used to amplify and simultaneously quantify one or more targeted sequences in a DNA molecule. Presence of those sequences is detected as the reaction progresses in real time, by measuring fluorescence after excitation with a laser; increased product causes increased fluorescence at each cycle. Amplified DNA theoretically doubles at every cycle during the exponential phase and relative amounts of DNA can be calculated by plotting fluorescence against the cycle number on a logarithmic scale and comparing the results to a standard curve produced by RT-PCR of serial dilutions of a known amount of DNA.

5.1.4 Amplification

PCR is an enzymatic process in which specific regions of DNA are replicated over and over again to yield many copies of particular sequences. This molecular 'xeroxing' process involves heating and cooling samples in a precise thermal cycling pattern for ~30 cycles. During each cycle, a copy of the target DNA sequence is generated for every molecule containing the target sequence. Theoretically, after 30 cycles approximately a billion copies of the target region on the DNA template will have been generated. In most forensic applications, fluorescently labeled primers are used to amplify and tag specific regions of DNA. The resulting PCR product is then of sufficient quantity, and in a detectable form for genetic analysis.

5.1.5 Detection

The PCR products, or fragments of DNA, must be separated and detected. Capillary electrophoresis (CE) is used to separate the fragments by size. The negatively charged phosphate groups of DNA cause migration away from the negative electrode and toward the positive electrode when an electric field is applied. A viscous polymer, contained within the capillary, acts as a sieving medium in which smaller molecules will pass more quickly. As the DNA fragments travel through the capillary they eventually reach a laser window where they are illuminated, which excites the fluorescent dyes attached to the fragments and light is emitted at a specific wavelength for each dye. The light is collected and separated according to wavelength. The data collection software collects the light intensities and stores them as electrical signals. Computer software is then used to analyze the collected data and present it for interpretation.

CE allows for automation of the sample injection, separation, and detection. The separations are fast, little sample is used, retesting is easy, and cross-contamination risk is reduced.

5.1.6 Documentation and Evidence Sample Selection

Once the appropriate components of DNA analysis have been successfully completed, it is important to be able to put them to use in conducting case analysis. Taking proper notes, correctly filling out worksheets, choosing the proper method of analysis, and carrying out each step while avoiding contamination are important during the analysis of case evidence and database samples.

The following measures must be taken to avoid contamination during analysis: properly clean implements, plates/tube racks, and workbench; use clean examination paper; separate question and known casework samples by time and/or space; use appropriate blanks and controls; and change gloves as necessary.

It is important to be organized, clear, and concise, while including important details during case documentation. It is also important to not lose sight of the overall details of the case while conducting each step of the analysis.

Proper avenues should be taken to obtain the information needed about the case to enable proper sample selection and analysis. One must be organized in the handling of information for each case and be able to properly organize the documentation in the form of a note packet and report. The report of analysis will reflect results for only the sample(s) selected for testing, without making an inference about the entire population.

5.2 Objectives

To gain the necessary knowledge base and mastery of the technical skills needed to perform all aspects of DNA analysis. This includes, but is not limited to, the following: historical understanding of DNA analysis and its application in forensic science, theoretical and applied skills in DNA methodologies, familiarity with instrumentation, contamination awareness and use of 'clean technique', mixture interpretation, and relevant population genetics and statistical analyses. Upon successful completion of this module an analyst will be able to operate the necessary instrumentation (including performance/documentation of maintenance), perform, interpret, and communicate the results of DNA analyses, conduct critical reagent QC, and participate in research/validation relevant to the future development and improvement of DNA methodologies employed in the Biology Section.

5.3 Practical Exercises

Reading, observation of individual procedures (documented on the observation log), and the hands-on exercises listed below. All reading and observations must be completed prior to performing the corresponding exercises.

5.3.1 Casework

Exercises need not be performed in the order listed below. Retain all collected and/or prepared samples and extracts (store frozen) for use throughout the hands-on exercises.

5.3.1.1 Extraction

- Obtain known liquid blood samples from five different individuals and prepare two 5ul

bloodstains from each. Extract the entire stain on the Qiagen BioRobot EZ1(s) using a 50ul elution volume for one set and 200ul for the other.

- Prepare serial dilutions of two blood samples to 1:500 and make three 5ul stains for each dilution amount. Perform EZ1, organic and chelex extraction for each dilution.
- Perform EZ1 extraction on various body fluids (collected from a single individual as much as possible), included but not limited to blood, semen, aspermic semen, vaginal fluid, hair, saliva, urine, and feces. Compare liquid versus stains where applicable.
- Collect reference oral swabs from the mother, father, and child(ren) of at least three different families. Extract each on the EZ1(s).
- Prepare mixtures of the following body fluids collected from different individuals (male and female where appropriate), in ratios of 50:1, 25:1, 10:1, 1:1, 1:10, 1:25, and 1:50, by volume: blood:semen; saliva:semen; blood:blood; saliva:blood; semen:semen. Perform EZ1 extraction on each of the mixtures, using the differential extraction method on those samples containing semen (except semen:semen).
- Perform a differential extraction (organic and EZ1) on semen-stained vaginal swabs collected at varying post-coital intervals (to at least 48 hours).

5.3.1.2 Quantitation

- Using the current quantitation kit, prepare the standard dilution series. Perform a quantitation of these standards and print the standard curve. Compare the R^2 and slope to that of the kit QC/SRM 2372 results.
- Prepare a dilution series (similar to that of the quantitation kit standard) of the positive amplification control (2800M). Perform a quantitation of these dilutions and, without using the automated 7500 results form, perform the normalization calculations.
- Retrieve 10 previously quantitated extraction control samples and note the concentration listed for each. Re-quant each of these 10 samples and

compare results to those previously obtained. Manually calculate normalization dilutions for these samples. Import the results into the automated 7500 results form and re-calculate the normalization values using the form. Compare the results and note any differences.

- Prepare a written summary of all observations made. If differences are observed from known values (or previous results), discuss the significance and give possible reasons for the discrepancies, if known.

  Note: Samples extracted in 5.3.1.1 will also need to be quantitated (see below); however, they are independent of successful completion of this section.

- Perform quantitation and normalization calculations on each of the samples extracted in 5.3.1.1. Compare the concentration obtained from body fluids of the same individual, as well as duplicate samples extracted with different methods or different elution volumes.

5.3.1.3 Amplification

- Prepare a dilution series from 2ng – 0.03ng of 2800M or previously extracted and quantified DNA. Amplify each sample using the approved protocol.
- Prepare a dilution series of high concentration samples (may be previously extracted/quantified or newly extracted/quantified) from 10ng – 2.5ng. Amplify each using the approved protocol.
- Amplify each of the extracted/quantified samples listed in 5.3.1.1.

5.3.1.4 Capillary Electrophoresis and Data Analysis

- Using provided electropherograms (single source and mixtures) and sizing tables, manually genotype each sample, taking into account stutter and any other artifacts that may be present. Analyze the corresponding .FSA files in Genemapper ID. Compare the allele calls made.
- Perform Genetic Analyzer instrument setup (trap flush, water wash, buffer change, water change,

polymer change or replenish, and array change if necessary). Prepare and run a matrix/spectral calibration, as well as a spatial.

- Run the sensitivity dilution series amplified in 5.3.1.3 at 3 seconds, 5 seconds, and 10 seconds. Analyze the data and observe any stochastic effects/allele drop out and the lower limits of detection.
- Run the high concentrations samples amplified in 5.3.1.3. Analyze the data and note any -A, stutter, pull-up or other artifacts that may be present. Dilute amplified product and rerun as warranted.
- Run the single source bloodstains (50ul/200ul elution and dilution series) and analyze the data. Note any stochastic effects/allele dropout and calculate peak height ratios for heterozygous loci.
- Run the body fluid mixture samples, varying injection time as necessary. Analyze the data and note the levels at which a minor component can be detected. Perform mixture calculations, as appropriate, to aid in determining the most likely major and minor genotypes.
- Run the remainder of the samples amplified in 5.3.1.3, varying injection times as necessary. Analyze the data and perform mixture deconvolution as appropriate.

5.3.1.5 Statistics

- Perform statistical calculations for paternity on each of the family samples.
- Perform random match probability or mixture calculations (likelihood ratio or probability of inclusion/exclusion), as appropriate, for one set of body fluid mixtures and the post-coital samples.

5.3.1.6 Case Review

Review a minimum of ten case files (encompassing multiple sample types and analysts) to include note format/worksheets, evidence description, sketches/photographs, sample selection, statistics, report writing, and communication logs.

5.3.1.7 Mock Cases

Process a minimum of two mock DNA cases and write reports for each. The documentation and analysis of items are to be performed as would be done in the course of normal casework. A previously analyzed (or known results) proficiency test may be substituted for one of the mock cases.

Note: non-probative cases may be analyzed in addition to mock cases as available.

5.3.2 Database

5.3.2.1 Extraction

- Obtain buccal FTA samples from a minimum of 16 different individuals. Punch the samples using the BSD puncher and perform FTA extraction, manual DNA IQ extraction, and Automated Biomek DNA IQ extraction on each.
- Obtain 40 buccal FTA samples (may use duplicate samples if necessary). Punch the samples into a 'checkerboard' (i.e. samples alternating with blanks) plate pattern with the BSD puncher. Extract the plate using the Automated Biomek DNA IQ method.
- Obtain minimum of 23 buccal FTA samples (include 1 reagent blank). Punch each sample onto two separate plates with the BSD puncher. Perform Automated Biomek DNA IQ extraction with an elution volume of 100ul for one set and 50ul for the other.

5.3.2.2 Quantitation

- Using the current quantitation kit, prepare the standard dilution series. Perform a quantitation of these standards and print the standard curve. Compare the R^2 and slope to that of the kit QC/SRM 2372 results.
- Prepare a dilution series (similar to that of the quantitation kit standard) of the positive amplification control (2800M). Perform a quantitation of these dilutions and, without using

the automated 7500 results form, perform the normalization calculations.

- Retrieve 10 previously quantitated samples and note the concentration listed for each. Re-quant each of these 10 samples and compare results to those previously obtained. Manually calculate normalization dilutions for these samples. Import the results into the automated 7500 results form and re-calculate the normalization values using the form. Compare the results and note any differences.
- Prepare a written summary of all observations made. If differences are observed from known values (or previous results), discuss the significance and give possible reasons for the discrepancies, if known.

Note: Samples extracted in 5.3.2.1 will also need to be quantitated (see below); however, they are independent of successful completion of this section.

- Perform quantitation and normalization calculations on each of the samples extracted in 5.3.2.1 (Manual quantification setup is to be performed on the manual DNA IQ samples. FTA extracted samples will not be quantified). Compare the concentration obtained from duplicate samples extracted with different methods or different elution volumes.

5.3.2.3 Amplification

- Prepare a dilution series from 2ng – 0.03ng of 2800M or previously extracted and quantified DNA. Amplify each sample using the approved protocol.
- Prepare a dilution series of high concentration samples (may be previously extracted/quantified or newly extracted/quantified) from 10ng – 2.5ng. Amplify each using the approved protocol.
- Amplify each of the extracted/quantified samples listed in 5.3.2.1. Normalization and amplification setup of the manual DNAIQ plate will also be done manually.

5.3.2.4 Capillary Electrophoresis and Data Analysis

- Using provided electropherograms and sizing tables, manually genotype each sample, taking into account stutter and any other artifacts that may be present. Analyze the corresponding .FSA files in Genemapper ID. Compare the allele calls made.
- Perform Genetic Analyzer instrument setup (trap flush, water wash, buffer change, water change, polymer change or replenish, and array change if necessary). Prepare and run a matrix/spectral calibration, as well as a spatial.
- Run the sensitivity dilution series amplified in 5.3.2.3 at 3 seconds, 5 seconds, and 10 seconds. Analyze the data and observe any stochastic effects/allele drop out and the lower limits of detection.
- Run the high concentrations samples amplified in 5.3.2.3. Analyze the data and note any -A, stutter, pull-up or other artifacts that may be present. Dilute amplified product and rerun as warranted.
- Run the samples amplified in 5.3.2.3, varying injection times as necessary and analyze the data.

5.3.2.5 Previously Typed Offender Samples

Process two full plates of previously-typed offender samples. Include an extraction control and reagent blank on each plate. The documentation and analysis of samples are to be performed as would be done in the course of normal database analysis. Previously analyzed (or known results) proficiency test samples may be substituted for some of the offender samples.

5.3.3 Quality Control

- Perform critical reagents kit QC.
- Perform monthly maintenance/QC on instruments.
- Perform quarterly maintenance/QC on instruments.

5.4 Principles/Knowledge:

The analyst should have an understanding of DNA analysis, which may include but is not limited to the following:

- Fulfillment of Coursework Requirements (dictated in current national guidelines)
- Basic Forensic DNA Knowledge (e.g., terms, biochemistry, technological history, statistics/population genetics, and mixture interpretation)
- Understanding of DNA Methodologies (e.g., theory, mechanism, sensitivity, limitations, utility of controls, and safety hazards)
- Application of Biology Section Protocols for DNA analysis
- Assessing Biological Samples for DNA Analysis (Sample Selection Methods for Casework)
- Methods to Avoid Contamination
- Case File or Database Packet Content and Organization
- Report Writing and Communication
- Operation/Maintenance/Documentation of Instrumentation in the Biology Section

6.0 Module 6: Court: Decisions and Testimony

6.1 Background Information and Theory

Often times following the completion of an evidentiary examination and issuance of a report, the analyst is called to present those results to a jury. It is important not only for the analyst to understand the techniques employed and the interpretation of results, but to be able to explain them to a group of people who may not have a science background. This explanation is achieved through a series of questions and answers between the attorneys and the analyst. It is therefore important to become familiar with court proceedings and testimony etiquette in order to answer these questions factually, accurately, and concisely. The analyst must remember that he/she is presenting facts regarding the evidence and analytical results and should remain an unbiased witness throughout the proceedings.

6.2 Objectives

To introduce the trainee to the legal system in the state of Idaho, relevant case law regarding scientific testimony, and to acquire the knowledge base and skills necessary to become a competent expert witness.

6.3 Practical Exercises

Reading, observation, and mock courts, as outlined in the corresponding evaluation form.

6.4 Principles/Knowledge:

The analyst should have an understanding of courtroom testimony and procedures, which may include but is not limited to the following:

- Relevant Literature and Court Decisions
- Courtroom Procedures and Rules
- Proper Attire and Demeanor
- Responsibilities of an Expert Witness
- Court Calendar and Subpoenas
- Curriculum vitae (CV) Preparation
- Discovery Requests

7.0 Module 7: Combined DNA Index System (CODIS)

7.1 Background Information and Theory

The Combined DNA Index System (CODIS),established and funded by the Federal Bureau of Investigation (FBI), is a computer software program for the operation of local, state, and national DNA databases. Designed for the storage and searching of STR profiles, the software permits sharing and comparing of qualifying DNA profiles between participating labs. To ensure uniformity for the sharing of information, the FBI has chosen 13 core STR loci to serve as the standard for CODIS. A weekly search is conducted of all DNA profiles in the National DNA Index System (NDIS) and the submitting laboratories are notified of potential matches automatically.

Each state determines its own policies concerning samples allowed in the state database, via legislation regarding the database creation and use. The designated CODIS Administrator serves as the CODIS point of contact and as gatekeeper for the data from the state database into NDIS. Laboratories that contribute to NDIS must comply with NDIS Procedures, which includes rules that govern DNA profile uploads. Some sample categories and profiles that don't meet a minimum number of loci are not eligible for entry into NDIS. The eligible DNA categories or indexes include, but are not limited to, convicted offenders, forensic unknowns (from crime scene evidence), and missing persons.

Forensic profiles from both solved and unsolved cases are entered into the databases in an effort to assist law enforcement agencies in solving or linking crimes that may not have otherwise resulted in the identification of a suspect. The effectiveness of the databases lies in the concept that many crimes are committed by repeat offenders. As the databases grow in size the effectiveness will also increase.

7.2 Objectives

To gain the knowledge base and skills necessary to enter and maintain DNA profiles in CODIS. This will include but is not limited to the following: CODIS software operation and hierarchy, understanding sample eligibility requirements, security procedures, review requirements, accepted specimen categories and indexes, and minimum number of loci

requirements. Upon successful completion of this module, the trainee will be able to determine whether a given profile (including mixtures) may be entered/uploaded into the database and when, identify the core loci, enter samples through STR data entry and/or using the import function, and perform keyboard searches as necessary.

7.3 Practical Exercises

Reading, observation of individual procedures (documented on the observation log), and the supervised tasks listed below. All reading and observations must be completed, and the trainee must be approved to begin supervised DNA casework or independent database analysis, prior to performing the supervised tasks. Additionally, NDIS approval must be obtained prior to obtaining login access to the CODIS workstation.

- Enter a minimum of three profiles using STR data entry.
- Locate the specimens entered through STR data entry and print specimen details reports for review by the CODIS Administrator.
- Enter a minimum of two .cmf files using the import function.
- Perform a minimum of two keyboard searches.

7.4 Principles/Knowledge

The analyst should have an understanding of CODIS, which may include but is not limited to the following:

- Utility of DNA Database
- CODIS software
- NDIS eligibility
- Available specimen categories
- Review and documentation requirements prior to search and upload
- Security requirements

8.0 Module 8: Administrative and Technical Review

8.1 Background Information and Theory

Administrative and Technical reviews are an integral part the laboratory's quality assurance system and must be completed on 100% of biology/DNA cases and database packets to ensure the reports issued to customers and data uploaded to CODIS meet all of the laboratory's quality standards. It also ensures the conclusions made are scientifically reasonable and based upon the data obtained. In general the review will verify that all documentation (reports, note packets, worksheets, etc.) is accurate, legible, free of clerical errors and consistent with laboratory protocols. Some specific elements of the review will include, but is not limited to, the following: all testing was accurately documented in the notes and required worksheets; the data generated meets all of the required specifications; the casework report accurately reflects the evidence examined, testing performed, the analysts conclusions, and statistical calculations if appropriate; the evidence chain of custody has been completed; and the profiles to be entered into CODIS meet eligibility requirements. The administrative and technical review must be performed by a second qualified analyst and prior to the release of results and/or entry of data into CODIS.

8.2 Objectives

To gain the knowledge and skills necessary to perform administrative and technical reviews of biology/DNA case files or database packets. Upon successful completion of this module, the trainee will understand the conflict resolution process, elements of both administrative and technical review, proper file/packet organization, and how to properly document the review.

The trainee must have been previously approved to perform and have gained experience performing independent casework or database analysis, as well as received review feedback of his/her cases/database packets prior to beginning this module.

8.3 Practical Exercises

Reading, and mock administrative/technical reviews of case files or database packets. The trainee will perform mock administrative and technical review by flagging the file or packet with a note indicating the specific concern or question. No notations will be made directly on the note packet by the trainee. The flagged file or packet will be given to a qualified analyst/reviewer for the official review. The qualified reviewer will evaluate the trainee's notes and discuss any discrepancies in findings observed.

8.4 Principles/Knowledge

The analyst should have an understanding of the process and requirements of administrative and technical review, which may include but is not limited to the following:

- Documentation of Review (notations/worksheets)
- Conflict Resolution Process
- Documentation of Corrections Made to Files
- Case File Organization