

Idaho State Police Forensic Services



Biology/DNA Training Manual

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Revision History

Revision #	Description of Changes
1	Original issue in new template. Removed references to evidence tracking system, DNA packets, Chelex extraction, organic extraction, DNA IQ, and Biomek. Added QIAcube, EZ1 for DNA database, A2LA, supervised database plates, and acceptance criteria. Updated required reading and # of CODIS core loci. Replaced references to training evaluation form with Qualtrax, Genemapper ID with analysis software, and specific statistical calculations (RMP, LR, CPI/CPE) with approved protocol. Corrected clerical errors.
2	Expanded DNA casework interpretation training including 3 and 4 person mixtures
3	Added Y-screen and minimum #s of supervised cases/plates. Updated to allow for Qualtrax or other approved method for documenting training. Corrected clerical errors
4	Converted to pdf following automated conversion system error. No other changes were made.
5	Added checklists to Appendix and removed Reading and Practical Exercises sections from body of manual. Removed modular system structure. Removed references to creatinine in serology sections. Updated checklists including: added Y-screen mock data analysis, hair analysis practical exercises, and minimum numbers to hands of the analyst training, removed obsolete readings.

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1.0 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 multiple sections 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 sections may be supplemented with technical lectures provided by qualified analysts. Progress of the trainee will be monitored either through the use of the Qualtrax software program, in which successful completion of a section is indicated electronically by the trainee, trainer, and/or technical lead, or other appropriate documentation method as approved by the Quality Manager. 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 sections 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 lead. In some instances, it may be sufficient for the experienced analyst to simply complete a competency test prior to beginning analysis.

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

The acceptance criteria for verbal/written examinations will require a minimum passing score of 80%. Practical exercises and competency tests will be reviewed by the trainer and/or technical lead and a pass/fail determination will be made. The trainee should demonstrate the appropriate knowledge, skills, and abilities relevant to the practical exercise in order to pass. To pass a competency test the trainee should obtain the expected results with no unexplainable discrepancies. If the

Biology/DNA Training Manual Introduction

Il Revision 5 Issue Date: 01/06/2021 Page 6 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled acceptance criteria are not met for a verbal/written examination, practical exercise, or competency test, then remediation will be required prior to progressing in the training program. The required remediation will be determined by the trainer and/or technical lead and may take a variety of forms including additional training assignments, exercises, examinations, and/or competency tests, as appropriate.

When completing a training checklist, it is expected that all readings and observations will be completed prior to performing the corresponding exercises.

Approval to perform supervised cases must be obtained from the Quality Manager. Supervised casework will only be performed after completion of all other requirements of the associated training checklist.

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2.0 Roles and Responsibilities

- 2.1 Technical Lead The unit technical lead 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 lead should regularly monitor the trainee's progress and review their training binder for completeness and accuracy. At a minimum, the technical lead should meet with the trainee at the end of each section to discuss the exercises and any further actions.
- 2.2 Trainer The trainer will be the unit technical lead 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 trainee/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 section and/or training program. The trainer's electronic approval in Qualtrax and/or initials on 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 lead prior to approval. In addition, Supervised Casework and Technical Review logs will be completed by the trainer. The trainer, if not the technical lead, shall periodically meet with the technical lead to discuss the progress of the trainee.

2.3 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 electronic documentation in Qualtrax or other approved documentation method indicates 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 lead informed as to their progress and/or any problems or questions that may arise. The trainee

Biology/DNA Training Manual Roles and Responsibilities

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3.0 Laboratory Introduction

- 3.1 Background and Theory
 - 3.1.1 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, 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.
 - 3.1.2 Beginning in 1987, all three laboratories were accredited by the American Society of Crime Laboratory Directors - Laboratory Accreditation Board (ASCLD-LAB). In 2017 the laboratories accreditation transitioned to the American Association for Laboratory Accreditation (A2LA). 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.
 - 3.1.3 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

Biology/DNA Training Manual Laboratory Introduction

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- 3.2 Objectives, Principles, and Knowledge
 - 3.2.1 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.
 - 3.2.2 The analyst should have an understanding of ISP and the Forensic Services laboratory, which may include but is not limited to the following:
 - 3.2.2.1 Orientation to Laboratory/ISP
 - 3.2.2.2 Organizational Structure, Chain of Command, and Policies and Procedures
 - 3.2.2.3 Security and Confidentiality Issues (e.g., access codes, visitors, communications, and data privacy)
 - 3.2.2.4 Quality Assurance/Quality Control
 - 3.2.2.5 Health and Safety Guidelines (e.g., universal precautions, bloodborne pathogens, chemical hygiene, biohazard disposal, decontamination, and vaccinations) *Note: Safety hazards associated with specific analytical methods and/or instruments will be covered as part of the associated section.*
 - 3.2.2.6 Location and Use of Safety Equipment
 - 3.2.2.7 Introduction to Other Forensic Disciplines
 - 3.2.2.8 Ethics in Forensic Science
- 3.3 Health and Safety Hazards
 - 3.3.1 N/A
- 3.4 Reading and Practical Exercises Refer to the Introduction, Evidence Handling, and Testimony Training Checklist.

4.0 Evidence/Offender Sample Handling

- 4.1 Background and Theory
 - 4.1.1 Evidence Items
 - 4.1.1.1 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 evidentary requests and needs. All of the examinations, analyses conducted, and samples collected must be properly documented.
 - 4.1.1.2 All evidence submitted to the laboratory is entered into a 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.
 - 4.1.2 Offender Samples
 - 1.2.1 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.
 - 4.1.2.2 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.

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- 4.1.2.3 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.
- 4.2 Objectives, Principles, and Knowledge
 - 4.2.1 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.
 - 4.2.2 The analyst should have an understanding of evidence or offender sample handling, which may include but is not limited to the following:
 - 4.2.2.1 Case/Evidence/Offender Sample Acceptance and Receipt
 - 4.2.2.2 Computer System (e.g., LIMS, Tracker)
 - 4.2.2.3 Chain of Custody Maintenance/Documentation
 - 4.2.2.4 Evidence/Offender Sample Packaging and Storage
 - 4.2.2.5 Evidence Handling, Retention/Consumption, and Documentation
 - 4.2.2.6 Case Assignment (backlog and tracking procedures)
- 4.3 Health and Safety Hazards
 - 4.3.1 Biological evidence and offender samples are handled in accordance with "Universal Precautions".
- 4.4 Reading and Practical Exercises

Casework - Refer to the Introduction, Evidence Handling, and Testimony Training Checklist.

Database - Refer to the Database Offender Sampling Training Checklist.

5.0 Supplies, Quality Control, and Reagents

- 5.1 Background and Theory
 - 5.1.1 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.
 - 5.1.2 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.
 - 5.1.3 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.
- 5.2 Objectives, Principles, and Knowledge
 - 5.2.1 To gain familiarity with ordering of supplies, reagents, and quality control in the Biology Section. After completion of this section the employee should be able to make reagents, order supplies/chemicals, and perform/document routine quality control checks.
 - 5.2.2 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:
 - 5.2.2.1 Reagent Preparation and Log
 - 5.2.2.2 Purchasing Card Training
 - 5.2.2.3 Ordering and Documentation
 - 5.2.2.4 Chemical Inventory
 - 5.2.2.5 MSDS and Safe Handling/Use of Chemicals/Reagents

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- 5.2.2.6 Labeling and Documentation for Supplies and Chemicals/Reagents Made or Received in the Biology Section
- 5.2.2.7 Quality Control Checks
- 5.3 Health and Safety Hazards
 - 5.3.1 See SDS and/or product inserts for health and safety hazards associated with specific chemicals/reagents.
- 5.4 Reading and Practical ExercisesRefer to the Supplies, Quality Control, and Reagents Training Checklist.



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6.0 Biological Screening

- 6.1 Background and Theory
 - 6.1.1 Blood Tests
 - 6.1.1.1 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.
 - 6.1.1.2 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.
 - 6.1.2 Semen Tests
 - 6.**1**.2.1 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

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Il Revision 5 Issue Date: 01/06/2021 Page 16 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled 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.

- 6.1.2.2 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 picroinligo 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.
- 6.1.2.3 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. 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.
- 6.1.3 Saliva Test

6.1.3.1 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.

- 6.1.4 Urine Tests
 - 6.1.4.1 Like saliva, urine is difficult to conclusively identify because of the lack of sufficient amounts of substances unique to urine. Urea is a

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Il Revision 5 Issue Date: 01/06/2021 Page 17 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled substance found in high concentrations in urine but can also be found, at lower concentrations, in other fluids including: blood, perspiration, saliva and semen.

- 6.1.4.2 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.
- 6.1.4.4 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.
- 6.1.5 Feces Test
 - 6.1.5.1 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. Urobilizogen 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.
 - 6.1.5.2 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.

6.1.6 Y-Screen

6.1.6.1 The Y-screen method for sexual assault evidence collection kit examination involves the detection of male DNA using a DNA quantitation method where both autosomal and male DNA are targeted. This method is useful in sexual assault cases with a female victim and male perpetrator(s). This method can be more efficient and sensitive than traditional serological approaches to screening sexual assault kits.

- 6.1.7 Alternate Light Source
 - 6.1.7.1 Dried stains of physiological fluids such as semen, saliva, and urine can be difficult to locate on an item of evidence under normal

Biology/DNA Training Manual Biological Screening

Il Revision 5 Issue Date: 01/06/2021 Page 18 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled 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 450nm. 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.

- 6.1.8 Infra-Red (IR)
 - 6.1.8.1 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.

6.1.9 Evidence Examination/Sample Selection

6.1.9.1 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

Biology/DNA Training Manual Biological Screening

Il Revision 5 Issue Date: 01/06/2021 Page 19 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled 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.

- 6.1.9.2 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.
- 6.2 Objectives, Principles, and Knowledge
 - 6.2.1 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 section, an analyst will be able to perform casework processing up to and including the creation of a DNA assignment, 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.
 - 6.2.2 The analyst should have an understanding of biological evidence screening, which may include but is not limited to the following:
 - 6.2.2.1 Evidence Acceptance Policies and Testing Schemes
 - 5.2.2.2 Understand Screening Tests (e.g., theory, mechanism, sensitivity, limitations, various techniques, utility of controls, and safety hazards)
 - 6.2.2.3 Application of Biology Protocols for the Identification of Body Fluids
 - 6.2.2.4 Methods of Evidence Documentation
 - 6.2.2.5 Methods to Avoid Contamination
 - 6.2.2.6 Sample Selection Methods and Conclusions
 - 6.2.2.7 Case File Content and Organization
 - 6.2.2.8 Report Writing and Communication

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6.2.2.9 Quality Control

- 6.3 Health and Safety Hazards
 - 6.3.1 Blood and body fluids are handled in accordance with "Universal Precautions".
 - 6.3.2 See SDS and/or product inserts for health and safety hazards associated with specific chemicals/reagents.
 - 6.3.3 See relevant section of the ISP Forensic Biology Casework Analytical Methods for health and safety hazards associated with a specific analytical method.
 - 6.3.4 Alternate light source safety training shall be completed prior to use of the alternate light source.
- 6.4 Reading and Practical Exercises

Refer to the following Training Checklists, as applicable: Traditional Screening, Y-Screening.

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.



7.0 DNA Analysis

- 7.1 Background and Theory
 - 7.1.1 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).
 - 7.1.2 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.
 - 7.1.3 History
 - 7.1.3.1 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 for ensic biology to address these limitations.

7.1.3.2 PCR has been successful in yielding genetic information from degraded samples and those of limited quantity. Early commercial kits (AmpliType HLA DQ- α , 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.

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7.1.4 Extraction

- 7.1.4.1 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.
- 7.1.4.2 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.
- 7.1.4.3 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.
- 7.1.4.4 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.
- 7.1.4.5 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.
 7.1.4.6 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. Additionally, some DNA typing kits are optimized for direct amplification of samples on FTA paper without requiring purification.
- 7.1.5 Quantitation

7.1.5.1 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

Biology/DNA Training Manual DNA Analysis

Il Revision 5 Issue Date: 01/06/2021 Page 23 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled 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.

- 7.1.5.2 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.
- 7.1.6 Amplification
 - 7.1.6.1 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.
- 7.1.7 Detection

7.1`

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

Biology/DNA Training Manual DNA Analysis

Il Revision 5 Issue Date: 01/06/2021 Page 24 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled 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.

- 7.1.7.2 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.
- 7.1.8 Interpretation
 - 7.1.8.1 Following development of a DNA profile the results must be interpreted. Interpretation includes evaluating the DNA profile to determine which peaks are alleles vs. artifacts, the number of contributors to the DNA profile, whether or Not a mixture profile can be broken down into major minor components, comparison to reference samples from known individuals, statistical calculations, and determination of a CODIS eligible profile. Statistical calculations that have been used include random match probability (RMP), combined probability of inclusion/exclusion (CPI/CPE), and likelihood ratio (LR). Software programs have been developed to aid in the interpretation process and calculation of statistics.
- 7.1.9 Documentation and Evidence Sample Selection
 - 7.1.9.1 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.
 - A 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.
 - 7.1.9.3 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.

Biology/DNA Training Manual DNA Analysis

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- 7.1.9.4 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.
- 7.2 Objectives, Principles, and Knowledge
 - 7.2.1 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 section 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.
 - 7.2.2 The analyst should have an understanding of DNA analysis, which may include but is not limited to the following:
 - 7.2.2.1 Fulfilment of Coursework Requirements (dictated in current national guidelines)
 - 7.2.2.2 Basic Forensic DNA Knowledge (e.g., terms, biochemistry, technological history, statistics/population genetics, and mixture interpretation)
 - 2.2.3 Understanding of DNA Methodologies (e.g., theory, mechanism, sensitivity, limitations, utility of controls, and safety hazards)
 - 2.2.4 Application of Biology Section Protocols for DNA analysis
 - 7.2.2.5 Assessing Biological Samples for DNA Analysis (Sample Selection Methods for Casework)
 - 7.2.2.6 Methods to Avoid Contamination
 - 7.2.2.7 Case File or Database Packet Content and Organization
 - 7.2.2.8 Report Writing and Communication
 - 7.2.2.9 Operation/Maintenance/Documentation of Instrumentation in the Biology Section
- 7.3 Health and Safety Hazards

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- 7.3.1 Blood and body fluids are handled in accordance with "Universal Precautions".
- 7.3.2 See SDS and/or product inserts for health and safety hazards associated with specific chemicals/reagents.
- 7.3.3 See relevant section of the ISP Forensic Biology Casework Analytical Methods for health and safety hazards associated with a specific analytical method.
- 7.4 Reading and Practical Exercises

Casework – Refer to the following Training Checklists, as applicable: Extraction, Quantification, Amplification, Capillary Electrophoresis and Data Interpretation, Statistics and Case Write Up.

Database – Refer to the Database Analyst Training Checklist. NOTE: Be sure to retain training samples until all checklists are completed, as some may be used during multiple training exercises.

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8.0 Court – Decisions and Testimony

- 8.1 Background and Theory
 - 8.1.1 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.
- 8.2 Objectives, Principles, and Knowledge
 - 8.2.1 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.
 - 8.2.2 The analyst should have an understanding of courtroom testimony and procedures, which may include but is not limited to the following:
 - 8.2.2.1 Relevant Literature and Court Decisions
 - 8.2.2.2 Courtroom Procedures and Rules
 - 8.2.2.3 Proper Attire and Demeanor
 - 8.2.2.4 Responsibilities of an Expert Witness
 - 8.2.2.5 Court Calendar and Subpoenas
 - 8.2.2.6 Curriculum vitae (CV) Preparation
 - 8.2.2.7 Discovery Requests
- 8.3 Health and Safety Hazards 8.3.1 N/A
- 8.4 Reading and Practical Exercises Refer to the Introduction, Evidence Handling, and Testimony Training Checklist.

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9.0 Combined DNA Index System (CODIS)

- 9.1 Background and Theory
 - 9.1.1 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 20 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.
 - 9.1.2 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.
 - 9.1.3 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.
- 9.2 Objectives, Principles, and Knowledge
 - 9.2.1 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 section, 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

Biology/DNA Training Manual Combined DNA Index System (CODIS)

Il Revision 5 Issue Date: 01/06/2021 Page 29 of 89 Issuing Authority: Quality Manager All printed copies are uncontrolled samples through STR data entry and/or using the import function, and perform keyboard searches as necessary.

- 9.2.2 The analyst should have an understanding of CODIS, which may include but is not limited to the following:
 - 9.2.2.1 Utility of DNA Database
 - 9.2.2.2 CODIS software
 - 9.2.2.3 NDIS eligibility
 - 9.2.2.4 Available specimen categories
 - 9.2.2.5 Review and documentation requirements prior to search and upload
 - 9.2.2.6 Security requirements
- 9.3 Health and Safety Hazards
 - 9.3.1 N/A
- 9.4 Reading and Practical Exercises
 Refer to the CODIS Training Checklist.
 NOTE: NDIS approval must be obtained prior to obtaining login access to the CODIS workstation.

10.0 Administrative and Technical Review

10.1 Background and Theory

- 10.1.1 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 aboratory 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.
- 10.2 Objectives, Principles, and Knowledge
 - 10.2.1 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 section, 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.
 - 10.2.2 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 section.
 - 10.2.3 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:

Biology/DNA Training ManualRevision 5Administrative and TechnicalIssue Date: 01/06/2021ReviewPage 31 of 89Issuing Authority: Quality ManagerAll printed copies are uncontrolled

10.2.3.1 Documentation of Review (notations/worksheets)

10.2.3.2 Conflict Resolution Process

10.2.3.3 Documentation of Corrections Made to Files

10.2.3.4 Case File Organization

10.3 Health and Safety Hazards

10.3.1 N/A

10.4 Reading and Practical Exercises

Casework – Refer to the following training checklists, as applicable: Serology Technical Review, STR Technical and Batch Review, YSTR Technical and Batch Review.

Database – Refer to the Database Review Training Checklist

The trainee will perform mock administrative and technical review by flagging the file or packet with a comment or 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 comments or notes and discuss any discrepancies in findings observed.



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11.0 Appendix A – Training Checklists

Introduction, Evidence Handling, and Testimony Training Checklist

Analyst:	
Primary Trainer Assigned:	
Technical Lead Plan Approval:	
Comments:	
Laboratory Introduction	Date Completed
Complete the ISPFS Core Training. A copy of the training sign-off documentation is to be included in the analyst's training file. Written examinations completed as	
part of Core Training will serve as written competency for this section.	
ISP Strategic Plan	
ISP Forensic Biology Quality Manual (current revision)	

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Appendix A – Training Checklists

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Court - Decisions and Testimony

Complete the ISPFS Core Training. A copy of the training sign-off documentation is to be included in the analyst's training file. Written examinations completed as part of Core Training will serve as written competency for this section.

Idaho State Judiciary, Idaho Court Rules: Idaho Rules of Civil Procedure, Idaho Criminal Rules, Idaho Rules of Evidence. <u>http://www.isc.idaho.gov/rulestxt.htm</u>

Koehler, J.J. Error and Exaggeration in the Presentation of DNA Evidence at Trial. Jurimetrics Journal, 1993; 34:21-39.

Prepare curriculum vitae, review with trainer.

Observe analyst(s) testimony (as available).

Attend a testimony course (as available).

Trainer to review mock court questions with trainee prior to mock court testimony. Trainer will provide feedback regarding responses and practice with trainee.

Mock court - to cover the extent of casework participation. Will be covered as part of first training checklist assigned.

Evidence/Offender Sample Handling

Complete the ISPFS Core Training. A copy of the training sign-off documentation is to be included in the analyst's training file. Written examinations completed as part of Core Training will serve as written competency for this section.

Kaye, J.A. Correspondence About Handling Evidence in Cases of Acquired Immune Deficiency Syndrome (AIDS) [letter]. American Journal of Forensic Medicine and Pathology, March, 1986; 7(1):87-88.

Successful completion of training and competency:

Trainer Signature Biology/DNA Training Manual Introduction, Evidence Handling, and Testimony Training Checklist / ____

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Date Completed

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	/		
Technical Leader Signature		Date	
Y - Sci	reening Traini	ng Checklist	
Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:			
Comments:			
Populings			Data Completed
Review relevant screening sections	of the ISP Forensic Bic	logy Casework Analytical	Date completed
Methods (current revision)			
Review relevant documentation and Quality Manual (current revision)	reporting section of	the ISP Forensic Biology	
Plexor [®] HY System for the Applied B Systems Technical Manual	Biosystems 7500 and 7	500 FAST Real-Time PCR	
Applied Biosystems 7300/7500/750 Maintenance Guide	0 Fast Real-Time PCR	System Installation and	
Linch, C.A.; Smith, S.L. and Prahlow, DNA Typing Subsequent to Microsco March 1998; 43(2):305-314.	J.A. Evaluation of the opic Comparison. Jour	Human Hair Root for nal of Forensic Sciences,	
Biology/DNA Training Manual Y - Screening Training Checklist	Page 35 of 89	Revision 5 Issue Date: 01/06/2021 Issuing Authority: Qual	L ity Manager

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Readings Continued			Date Completed
Mann, M.J. Hair Transfers in Sexual As	ssault: a Six-year Case	e Study. Journal of	
P00@108@13@00118@\$118118/18991019151(H&1952	waluation for DNA Ar	halysis" from West	
SwabSolution [™] Kit Technical Manual	(revision 09/16), Pror	nega Corporation.	
Practical Exercises/Discussions at Texa 2015.	as DPS" (PowerPoint	presentation), August	Date Completed
Examine at least 5 hairs (to include at SRESE Yalid, tigingfooth Scheener Comic Sibservations, photograph each, and p	least one animal and ને જે	l one human anagen နားစွဲတွော် နေတဲ့မင်္ကျန်နေနာ့မါး al imaging system.	
LERES EVENSED ALVALIABION AUBRC	K ହ୍ରସାନେଶ Brotsceletନା	hten Rapid Processing of	
Observe at least 2 different analysts p	process Y-screen batc	h from start to finish.	
Using the current quantitation kit, pre quantitation of these standards and p slope to that of the kit QC/SRM 2372	epare the standard di print the standard our results.	lution series. Perform a ve. Compare the r ² and	
Prepare a dilution series (similar to th positive amplification control (2800M	at of the quantitation). Perform a quantita	h kit standard) of the tion of these dilutions.	
Prepare swabs for the following samp without sperm), male saliva dilution s without sperm), male saliva on vagina biological fluids, and simulated case t the Y-screen method in at least two se	eries, semen dilut eries, semen on vagin al swabs, post-coital s ype samples. Test ea eparate batches.	ion series (with and nal swabs (with and wabs, female only ch of the swabs using	
Review a minimum of 10 Y-screen cas one positive hair case.	se files (various analys	sts), to include at least	
Complete mock data analysis exercise	e – minimum of 5 case	es.	
Hands of the Analyst: process a minim under constant observation by trained appropriate spreadsheet use and cutt	num of 2 Y-screen bar r, trainer signs report ing to DNA procedur	tches (5 cases each) s. Training to include es.	
Process a minimum of two mock y-scr The documentation and analysis of ite the course of normal casework.	reening cases and wri ems are to be perforr	te reports for each. ned as would be done in	
Biology/DNA Training Manual Y - Screening Training Checklist	Page 36 of 89	Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quali	ty Manager

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Quality Control		Date Completed
Perform critical reagents kit QC of the	SwabSolution kit.	
Perform critical reagents kit QC of the	AmpSolution kit.	
Perform critical reagents kit QC of the	Plexor HY kit.	
Competency		Date Completed
Complete and pass written exam.		
Complete and pass practical competer	псу.	
Testimony		Date Completed
Mock Court - to cover the extent of ca	sework participation.	
Supervised Cases Process a minimum of 10 Y-screen kits trainer; trainee signs reports.	s in at least two batches under supervision of	Date Completed
Successful completion of training and o	competency:	
Trainer Signature	/ Date	
Technical Leader Signature	/ Date	
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Traditional Screening Training Checklist

Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:			
Comments:			
Readings			Date Completed
Review relevant screening sections Methods (current revision)	of the ISP Forensic Blo	logy Casework Analytical	
Review relevant documentation an Quality Manual (current revision)	d reporting section of t	he ISP Forensic Biology	
Relevant product inserts and/or tec	chnical manuals	•	
Bunker, Judith, Bloodstain Evidence Characteristics, Institute of Applied	Manual, Vpl.3: Identif Forensic Technology, I	ication Stain and Pattern Doje's Press, 1998.	
Sensabaugh, G., The Utilization of P Isozymes: Current Topics in Biologic and Other Applications. 1983, 137-2	elymorphic Enzymes in sal and Medical Resear 154.	n Forensic Science. ch Volume 11: Medical	
Forensic Science Handbook, Vol. I, S Biology, pp.527-552, Prentice-Hall,	Second Edition, Chapte 2002.	r 10: Modern Forensic	
Forensic Science Handbook, Vol. II, Semen and Other Body Fluids, pp.3	Second Edition, Chapte 29-399, Pearson Prenti	er 8: The Identification of ce-Hall, 2005.	
Sourcebook in Forensic Serology, In on identification of various body flu	nmunology and Bioche Iids), U.S. Department	mistry (relevant sections of Justice, NIJ, 1983.	
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Brauner, P. and Gallili, N. A Condom--the Critical Link in a Rape. Journal of Forensic Sciences, September 1993; 38(5):1233-1236.



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Bryson, C.K., Garlo, A.M. and Piner, S.C. Vaginal Swabs: endogenous and postcoital components. Journal of the Forensic Science Society, May-June 1989; 29(3): 157-171.

Cox, M. A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood. Journal of Forensic Sciences, September 1991; 36(5):1503-1511.

Duenhoelter, J.H.; Stone, I.C.; Santos-Ramos, R. and Scott, D.E. Detection of Seminal Fluid Constituents After Alleged Sexual Assault. Journal of Forensic Sciences, October 1978; 23(4):824-829.

Gabby, T.; Winkleby, M.A.; Boyce, W.T.; Fisher, D.L.; Lancaster, A. and Sensabaugh, G.F. Sexual Abuse of Children. The Detection of Semen on Skin. American Journal of Diseases of Children, June 1992; 146(6):700-703.

Hochmeister, M.N.; Budowle, B.; Rudin, O.; Gehrig, C.; Borer, U.; Thali, M. and Dirnhofer, R. Evaluation of Prostate-specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid. Journal of Forensic Sciences, September 1999; 44(5):1057-1060.

Kamenev, L., LeClercq, M. and Francois-Gerard, Ch. Detection of p30 antigen in sexual assault case material. Journal of the Forensic Science Society, July-August 1990; 30(4):193-200.

Sensabaugh, G.F. Isolation and Characterization of a Semen specific Protein from Human Seminal Plasma: a Potential New Marker for Semen Identification. Journal of Forensic Sciences, January 1978; 23(1):106-115.

Willott, G.M., An Improved test for the Detection of Salivary Amylase in Stains. Journal of the Forensic Science Society, October 1974; 14(4): 341-344.

Willott, G.M. and Crosse, M.A. The Detection of Spermatozoa in the Mouth. Journal of the Porensic Science Society, March-April 1986; 26(2): 125-128.

Auvdel, M.J. Amylase Levels in Semen and Saliva Stains. Journal of Forensic Sciences, April 1986; 31(2):426-431.

Chapman, R.L., Brown N.M., and Keating, S.M. The isolation of spermatozoa from sexual assault swab using Proteinase K. Journal of the Forensic Science Society, May-June 1989; 29(3): 207-212.

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Ferris, L.E. and Sandercock, J. The Sensitivity of Forensic Tests for Rape. Medicine and Law, 1998; 17(3): 333-350.

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Groth, N.A. and Burgess, A.W. Sexual Dysfunction During Rape. New England Journal of Medicine, October 6, 1977; 297(14):764-766.

Hook, S.M.; Elliot, D.A. and Harbison, S.A. Penetration and Ejaculation: Forensic Aspects of Rape. New Zealand Medical Journal, March 11, 1992; 105(929):87-89.

Johnston, S. et al. Validation Study of the Abacus Diagnostics ABAcard[®] Hematrace[®] Membrane Test for the Forensic Identification of Human Blood. Journal of the Canadian Society of Forensic Science, 2003; 36(3): 173-183.

Joshi, U.N.; Subhedar, S.K. and Saraf, D.K. Effect of Water Immersion on Seminal Stains on Cotton Cloth. Forensic Science International, January-February 1981; 17(1):9-11.

Kafarowski, E.; Lyon, A.M. and Sloan, M.M. The Retention and Transfer of Spermatozoa in Clothing by Machine Washing. Canadian Society of Forensic Science Journal, 1996; 29(1):7-11.

Keating, S.M. and Higgs, D.F. The Detection of Amylase on Swabs from Sexual Assault Cases. Journal of the Forensic Science Society, April-June 1994; 34(2):89-93.

Kipps, A.E. and Whitehead, P.H. The Significance of Amylase in Forensic Investigations of Body Fluids. Forensic Science, December 1975; 6(3):137-144.

Myers, J.R. and Adkins, W.K. Comparison of Modern Techniques for Saliva Screening. Journal of Forensic Sciences, July 2008; 53(4):862-867.

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Sato, K.; Tsutsumi, H.; Htay, H.H.; Tamaki, K.; Okajima, H. and Katsumata, Y. Identification of Human Urinary Stains by the Quotient Uric Acid/Urea Nitrogen. Forensic Science International, March 1990; 45(1-2):27-38.

Schiff, A.F. Reliability of the Acid Phosphatase Test for the Identification of Seminal Fluid. Journal of Forensic Sciences, October 1978; 23(4):833-844. Silverman, E.M. and Silverman, A.G. Persistence of Spermatozoa in the Lower Genital Tracts of Women. Journal of the American Medical Association, October 20, 1978; 240(17):1875-1877.

Spear, T. and Khoshkebari, N. Analysis of Old Biological Samples: A Study on the Feasibility of Obtaining Body Fluid Identification and DNA Typing Results. California Criminalistics Institute.

Sweet, D. and Shutler, G.G. Analysis of Salivary DNA Evidence from a Bite Mark on a Body Submerged in Water. Journal of Forensic Sciences, September 1999; 44(5): 1069-1072.

Tobe, S.S. et al. Evaluation of Six Presumptive Tests for Blood, Their Specificity, Sensitivity, and Effect on High Molecular- Weight DNA. Journal of Forensic Sciences, 2007; 52(1):102-109.

Practical Exercises/Discussions

Observe analyst(s) perform traditional screening on a minimum of 6 cases (at least 3 blood, 3 sement non Y-screen. To include at least one traditional SAK). Training to include appropriate spreadsneet use and cutting procedures.

Lecture/Discussion on theory and background of evidence examination (IR, ALS) and presumptive and confirmatory tests for biological fluids (blood, semen, saliva, urine, feces).

Review a minimum of 10 traditional screening case files (various analysts and sample types).

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Prepare serial dilutions of liquid blood to at least $\sim 1/10,000$ (previously prepared may be used). Place 50 µl of each dilution onto clean white cloth and allow to air dry. Test the 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).

Prepare serial dilutions of liquid semen to at least $\sim 1/10,000$ (previously prepared may be used). Place 50 µl of each dilution onto clean white cloth and allow to air dry. Test the semen dilutions with acid phosphatase working solution 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 scain and perform AP mapping on them.

Test a variety of "aged" semen stains with the acid phosphatase working solution, 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).

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.

Complete the alternate light source safety training.

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). To include both LEEDS and PoliLight.

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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 patters, and/or any effect from the substrate. To include both LEEDS and PoliLight.

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 lights using the stereomicroscope, taking notes on overall appearance of the stains.

Examine the previously prepared bloodstains on dark fabric with the available IR cameras under the different available settings. 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.

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 descriptive (i.e. few moderate, many, etc.) and/or numeric (i.e. #/200X and 1+ - 4+) terms. Additionally, note any other cells observed and rate any epithelial cells. Document one slide representing each of the 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 staining of any cells observed.

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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. A range of dilutions will be tested until a negative result is achieved.

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). A range of dilutions will be tested until a negative result is achieved.

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. Note: may need to take photographs for reviewer.

Evaluate the specificity of the Phadebas method by testing various body fluids and animal saliva, as available.

Obtain a fresh urine sample and prepare a dilution series to 1:500. Using the analytical methods for Urease, test each of these dilutions to determine the sensitivity of the method.

Evaluate the specificity of the Urease method by testing various body fluids and animal urine, as available.

The following samples are to be fested 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.

Hands of the Analyst process a minimum of 5 traditional screening cases (at least 1 blood, 1 semen - non Y-screen) under constant observation by trainer, trainer signs reports. Training to include appropriate spreadsheet use and cutting to DNA procedures.

Biology/DNA Training Manual Traditional Screening Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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Practical Exercises/Discussions Continued Date Completed 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. **Quality Control** Date Completed Perform critical reagents kit QC of p30 cards Perform critical reagents kit QC of Hematrace cards Date Completed Competency Complete and pass written exam. Complete and pass practical competency. Testimony Date Completed Mock Court - to cover the extent of casework participation. **Supervised Cases Date Completed** Process a minimum of 10 traditional screening cases (5 blood/other, 5 semen - non Y-screen) under supervision of trainer; trainee signs reports. Successful completion of training and competency: **Trainer Signature** Date Technical Leader Signature Date **Revision 5** Biology/DNA Training Manual Traditional Screening Training Issue Date: 01/06/2021 Checklist Page 46 of 89 Issuing Authority: Quality Manager

EZ1® Extraction Training Checklist

Analyst:		
Primary Trainer Assigned:		
Technical Lead Plan Approval:		
Comments:		
Readings		Date Completed
Review relevant facilities, evidence, equi sections of the ISP Forensic Biology/DNA	ipment, and documentation/reporting Quality Manual (current revision)	
Review relevant DNA sections of the ISP Methods (current revision)	Forensic Biology/DNA Casework Analytical	
EZ1 [®] Advanced User Manual		
Applicable Validations/Performance Ver	ifications, including cited literature	
Quality Assurance Standards for Forensio revision) and associated guidance docum	c DNA Testing Laboratories (FBI) (current nent.	
The Evaluation of Forensic D-VA Evidence Academy Press, Washington, D.C. (1996)	e (National Research Council), National).	
DNA Technology in Forensic Science (Na Academy Press, Washington, D.C. (1992)	tional Research Council), National).	
Scientific Working Group on DNA Analys Documents (current revisions).	is Methods (SWGDAM) Guidelines	
https://www.swgdam.org/publications		
Butler, J.M. Fundamentals of Forensic DI	NA Typing. Academic Press (2009).	
Biology/DNA Training Manual	Revision 5	

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EZ1[®] Extraction Training

Checklist

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Butler, J.M. Advanced Topics in Forensic DNA Typing: Interpretation. Academic Press (2011).

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Alford, R.L. and Caskey, C.T. DNA analysis in forensics, disease and animal/plant identification. Current Opinion Biotechnology, 1994; 5:29-33.

Graham, E.A.M. et al. Can Post-Mortem Blood Be Used for DNA Profiling After Peri-Mortem Blood Transfusion? International Journal of Legal Medicine, 2007; 121:18-23.

Horsman, K.M. et al. Forensic DNA Analysis on Microfluidic Devices: A Review. Journal of Forensic Sciences, 2007; 52(4): 784-798.

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Montpetit, S.A. et al. A Simple Automated Instrument for DNA Extraction in Forensic Casework. Journal of Forensic Sciences, 2005; 50(3):1-9.

Opel, K.L. et al. Evaluation and Quantification of Nuclear DNA from Human Telogen Hairs. Journal of Forensic Sciences, 2008;53(4):853-857.

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Phipps, M. and Petricevic, S. The Tendency of Individuals to Transfer DNA to Handled Items, Forensic Science International, May 2007; 168(2):162-168.

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Soares-Viera, J.A. et al. Y-STRs in Forensic Medicine: DNA Analysis in Semen Samples of Azoospermic Individuals. Journal of Forensic Sciences, 2007; 52(3):664-670.

Biology/DNA Training Manual EZ1[®] Extraction Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Date Completed

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Checklist

Spear, T. et al. Fingerprints & Cartridge Cases: How Often are Fingerprints Found on Handled Cartridge Cases and Can These Fingerprints Be Successfully Typed for DNA? California Criminalistics Institute.

Spear, T. et al. Summary of Experiments Investigating the Impact of Fingerprinting Processing and Fingerprint Reagents on PCRbased DNA Typing Profiles. California Criminalistics Institute.

vanOorschot, R.A.H. et al. Beware of the Possibility of Fingerprinting Techniques Transferring DNA. Journal of Forensic Sciences, 2005; 50(6):1-6.

Yoshida, K. et al. The Modified Method of Two-step Differential Extraction of Sperm and Vaginal Epithelial Cell DNA from Vaginal Fluid Mixed with Semen Forensic Science International, March 21, 1995; 72(1):25-33.

Wilson, M.R. et al Validation for Mitochondrial DNA Sequencing for Forensic Casework Analysis. International Journal of Legal Medicine, 1995; 108(2):68-74.

Practical Exercises/Discussions		Date Completed
Trainer discussion/demonstration regardi and sample storage locations.	ng sample spreadsheet, creating batches,	
Lecture/Discussion on extraction. To inclu	de: chemistry involved, method used	
Trainer demonstration of associated work	sheets.	
Observe at least two casework batches be	ing created for extraction.	
Observation of E21 differential, non-diff, a	and reference extraction procedures.	
instrument.	ver proper cleaning/maintenance of the	
Observe at least one hair extraction.		
Obtain known liquid blood samples from f 5ul bloodstains from each. Extract the ent using a 50ul elution volume for one set an	ive different individuals and prepare two ire stain on the Qiagen BioRobot EZ1(s) d 200ul for the other.	
Prepare serial dilutions of two blood samp each dilution amount. Perform EZ1 extrac	bles to 1:500 and make one 5ul stain for tion for each dilution.	
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Date Completed

Practical Exercises/Discussions Continued Date Completed Perform EZ1 extraction on various body fluids included but not limited to blood, semen, aspermic semen, vaginal fluid, hair, saliva, urine, and feces. Dry on substrates and extract using spin baskets where possible. As available, collect reference oral swabs from the mother, father, and child(ren) of at least three different families. Extract each on the EZ1(s). Alternatively, extract at least 10 reference samples. Obtain a minimum of three hair samples and perform EZ1 extraction for each 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, 1:2 and 1:50, by volume: blood:semen; saliva:semen; blood:blood; saliva:blood; semen:semen. Perform EZ1 extraction on each of the mixtures, dried on swabs d fabric, using the manual differential extraction method on those samples containing semen (except semen:semen). Perform a differential extraction (manual) on semen-stained vaginal swabs collected at varying post-coital intervals (to at least 48 hours). **Quality Control** Date Completed Perform monthly maintenance/QC on EZ1 Competency Date Completed Complete and pass written examination Complete and pa practical competency Testimony **Date Completed** Mock court/oral communication assessment

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Biology/DNA Training Manual EZ1[®] Extraction Training Checklist

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Supervised Cases

Date Completed

Minimum of 2 differential, 2 non-differential, and 1 reference batch (to include spin basket where applicable, at least 24 [final] samples each).



Biology/DNA Training Manual EZ1[®] Extraction Training Checklist

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Plexor® HY Quantification and Normalization Training Checklist

Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:			
Comments:			
Readings		Date	Completed
Review relevant facilities, evidence sections of the ISP Forensic Biology	e, equipment, and docu ı/DNA Quality Mandal	mentation/reporting current revision)	
Review relevant DNA sections of th Casework Analytical Methods (curr	ne ISP Forensic Biology rent revision)	'DNA	
Plexor [®] HY System for the Applied Real-Time PCR Systems Technical N	Biosystems 7500 and 7 Janual	500 FAST	
Applied Biosystems 7300/7500/75 Installation and Maintenance Suid	00 Fast Real-Time PCR : e	System	
Applicable Validations/Performance	e Verifications, includin	ng cited literature	
Krenke, B.E. et al. Developmental A Simultaneous Quantification of Tot International: Genetics, December	Validation of a Real-Tim tal Human and Male DN 2008; 3(1):14-21.	e PCR Assay for the IA. Forensic Science	
Valasek, M.A.; Repa. J.J. The Power Education, 2005; 29(3):151-159.	r of Real-Time PCR. Adv	rances in Physiology	
Swango, K.L. et al. A quantitative P Degradation in Forensic Samples. F 158(1):14-26.	CR Assay for the Assess Forensic Science Interna	sment of DNA ational, April 2006;	
Biology/DNA Training Manual Plexor® HY Quantification and Normalization Training	Page 53 of 89	Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Ma	nager

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Checklist



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Practical Exercises/Discussions

Date Completed

Trainer demonstration of associated worksheets.

Lecture/discussion on quantification. To include: quantification chemistry, associated software, and data analysis.

Observe at least 2 analysts performing quantification plate setup, data export, and data analysis.

Observe write-up for 3 drop at quant cases.

Observe write-up for 3 forward-for-YSTR cases. Trainer should review requirements for forwarding samples to YSTR testing with trainee.

Observe extract return for at least 3 cases.

Review a minimum of 10 stop at quant case files, including a minimum of 3 forward-to-YSTR cases. Should encompass reports from multiple analysis.

Using Plexor[®] HY, prepare the standard dilution series. Perform a quantitation of these standards and print the standard curve. Compare the r² and slope to that of the kit QC 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 training 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.

Perform quantitation and normalization calculations on each of the samples extracted for completion of the EZ1[®] Extraction Training Checklist (or similar samples as assigned by the trainer).

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Date Completed

Process a minimum of two mock DNA batches, with a minimum of 16 samples each, through quantification and analyze each to the extent of quantification analysis. 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 analyzed in addition to mock cases as available.

Write up 2 mock drop at quant cases.

Write up 2 mock forward-for-YSTR cases.	
Quality Control	Date Completed
Perform critical reagents kit QC (Plexor [®] HY System Kit).	
Perform monthly maintenance/QC on 7500.	
Perform annual maintenance/QC on 7500.	
Competency	Date Completed
Complete and pass written examination	
Complete and pass practical competency	
Testimony	Date Completed
Mock court/oral communication assessment	

Biology/DNA Training Manual Plexor® HY Quantification and Normalization Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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Supervised Cases	Date Completed
Perform quantification plate set up, data analysis, and normalization for a minimum of two batches, at least 16 samples each. Normalization must include both dilutions and concentrations – additional mock samples may be provided to cover this requirement as needed.	
Minimum of 10 stop at quant case write ups, including at least 3 forward-to-YSTR.	
Successful completion of training and competency:	
Trainer Signature	
Technical Leader Signature bate	

Biology/DNA Training Manual Plexor[®] HY Quantification and Normalization Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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PowerPlex® Fusion 5C Amplification Training Checklist

Analyst:	
Primary Trainer Assigned:	
Technical Lead Plan Approval:	
Comments:	
Readings	Date Completed
Review relevant facilities, evidence, equipment, and docum	pertation /reporting
sections of the ISP Forensic Biology/DNA Quality Manual (c	urrent revision)
Review relevant DNA sections of the ISP Forensic Biology/D	NA Casework Analytical
Methods (current revision)	
PowerPlex [®] Fusion System for Use on the Applied Biosyster	ns [®] Genetic Analyzers
	•
Applicable Validations/Performance Verifications, including	cited literature
GeneAmp [®] PCR System 9700 User's Manual	
PCR: The Polymerase Chain Reaction. BirkHauser Boston (1	994). Ch 1, 3, and 21.
Alford, R.L. et al. Rapid and efficient resolution of parentage	e by amplification
of short tandem repeats. American Journal of Human Gene	tics, 1994 55: 190-195.
Budowle, B. et al. STR Primer Concordance Study. Forensic	Science International,
December 15, 2001; 124(1):47-54.	
Edwards, M.C. and Gibbs, R.A. Multiplex PCR: advantages, or applications, PCR Meth, Appl. 1994; 3: S65-S75	development, and
Oestelik K et el Develenmentel Velidetien ef the Dever	
for Analysis of Casework and Reference Samples: A24-Locu	s Multiplex for New
Database Standards. Forensic Science International: Geneti	cs, 2014; 12: 69-76.
Biology/DNA Training Manual PowerPlex [®] Eusion 5C	Revision 5 Issue Date: 01/06/2021
Amplification Training Checklist Page 58 of 89	Issuing Authority: Quality Manager
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Scherczinger, C.A. et al. A Systematic Analysis of PCR Contamination. Journal of Forensic Sciences, 1999; 44(5):1042-1045.

Walsh, P.S.; Erlich, H.A. and Higuchi, R. Preferential PCR amplification of alleles: Mechanisms and solutions. PCR Meth. Appl. 1992; 1: 241-250.

Practical Exercises/Discussions

Lecture on amplification. To cover at minimum: chemistry and background, oPCR vs PCR, primer design.

Trainer demonstration of associated worksheets.

Observe at least 2 analysts performing amplification plate seture.

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 utilized during the EZ1 Extraction/Plexor[®] HY Quantification and Normalization Training Checklists (or similar samples assigned by trainer.)

Combine previously generated DNA extracts from different individuals to create mixtures of DNA from 2, 3, and 4 individuals at varying mixtures ratios and input amounts. Amplify each using the approved protocol.

Quality Control

Date Completed

Date Completed

Date Completed

Perform critical reagents kit QC (PowerPlex® Fusion System Kit).

Perform annual maintenance/QC on 9700.

Biology/DNA Training Manual PowerPlex[®] Fusion 5C Amplification Training Checklist

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Competency	Date Completed
Complete and pass written examination	
Complete and pass practical competency	
Testimony	Date Completed
Mock court/oral communication assessment	
Supervised Cases	Date Completed
Perform amplification of two batches (minimum of 32 samples each).	
Successful completion of training and competency:	
Trainer Signature	
Technical Leader Signature Date	

Biology/DNA Training ManualRevision 5PowerPlex® Fusion 5CIssue Date: 01/06/2021Amplification Training ChecklistPage 60 of 89Issuing Authority: Quality ManagerAll printed copies are uncontrolled

PowerPlex® Fusion 5C Capillary Electrophoresis and Data Interpretation Training Checklist

Analyst: _____

Primary Trainer Assigned: _____

Technical Lead Plan Approval: _____

Readings

Date Completed

Review relevant facilities, evidence, equipment, and documentation reporting sections of the ISP Forensic Biology/DNA Quality Manual (current revision)

Review relevant DNA sections of the ISP Forensic Biology/DNA Casework Analytical Methods (current revision)

PowerPlex[®] Fusion System for Use on the Applied Brosystems[®] Genetic Analyzers Technical Manual

Applied Biosystems® 3130/3130xI Genetic Amalyzers Users Guide

Applicable Validations/Performance Verifications, including cited literature

Allor, C. et al. Identification and Characterization of Variant Alleles at CODIS STR Loci. Journal of Forensic Sciences, 2005; 50(5):1-6.

Budowle, B. et al. Population Data on the Thirteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. Journal of Forensic Sciences, November, 1999; 44(6): 1277-1286

Buel, E. et. al. Capillary Electrophoresis STR Analysis: Comparison to Gel-Based Systems. Journal of Forensic Sciences, 1998; 43(1):164-170.

Biology/DNA Training Manual PowerPlex® Fusion 5C Capillary Electrophoresis and Data Interpretation Training Checklist

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Date Completed

Butler, J.M. Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing. Journal of Forensic Sciences, 2006; 5(2):253-265. Butler, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual internal standards for precise sizing of Bolton, J.M. et al. Application of dual standards for precise sizing of Bolton, J.M. et al. Application of dual standards for precise sizing of the standards for precis	
Chakraborty, R. et al. The utility of short tandem repeat loci beyond human identification: Implications for development of new DNA typing systems. Electrophoresis, 1999 20(8):1682-1696.	
Gusmao, L. et al. DNA Commission of the International Society of Forensic Genetics (ISFG): An Update of the Recommendations on the Use of Y-STRs in Forensic Analysis. International Journal of Legal Medicine, 2006; 120(4):191-200.	
Fowler, J.C. et al. Repetitive Deoxyribonucleic Acid (DNA) and Human Genome Variationa Concise Review relevant to Forensic Biology. Journal of Forensic Sciences, 1988; 33():1111-1126.	
Mansfield, E.S. et al. Sensitivity, reproducibility, and accuracy in short tandem repeat genotyping using capillary array electrophoresis. Genome Research, 1996; 6: 893-903.	
Soborino, B. et al. SNPs in Forensic Genetics: A Review on SNP Typing Methodologies. Forensic Science International. November 2005; 154(2); 181-194.	
Wilson, M.R. et al Guidelines for the Use of Mitochondrial DNA Sequencing in Forensic Science. Crime Laboratory Digest, 1993; 20(4):68-77. Comments:	

Biology/DNA Training Manual PowerPlex® Fusion 5C Capillary Electrophoresis and Data Interpretation Training Checklist

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Practical Exercises/Discussions	•	Date Completed
Lecture on capillary electrophoresis. To cover at background, troublesbooting, instrument comp data interpretation.	t minimum: chemistry and onents and functions, and basic	
Trainer overview of GeneMapper functionality.		
Trainer demonstration of associated worksheet compiling.	s and batch documentation	
Observe at least 2 analysts performing capillary loading. To cover import file generation and cre	electrophoresis plate setup and ating multiple injections for run.	
Biology/DNA Training Manual PowerPlex [®] Fusion 5C Capillary	Revision 5 Issue Date: 01/06/2021	

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Electrophoresis and Data Interpretation Training

Checklist

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Practical Exercises/Discussions Continued	Date Completed
Observe at least 2 analysts performing batch documentation generation and data interpretation for batch review purposes.	
Review at least 5 batch documentation packets and ser projects from at least 3 different analysts. Should include a variety of batch types.	
Perform Genetic Analyzer instrument setup (trap flush, water wash, buffer change, water change, polymer change or replenish, and array change if necessary. Trainer should review processes at minimum).	
Run the sensitivity dilution series amplified for completion of the PowerPlex [®] Fusion 5C Amplification Training Checklist at 3 seconds, 5 seconds, and 10 seconds, (or similar samples as assigned by trainer). Analyze the data and observe any stochastic effects/allele drop out and the lower limits of detection.	
Run the high concentrations samples amplified for completion of the PowerPlex®	
trainer). Analyze the data and note any –A, stutter, pull-up or other artifacts that may be present. Dilute amplified product and rerun as warranted.	
Testimony Run the single source bloodstains (50ul/200ul elution and didution series) and	Date Completed
effects/allele dropout and calculate peak height ratios for heterozygous loci.	
Run the body fluid mixture samples, varying injection time as necessary (or similar	
samples as assigned by trainer). Analyze the data and note the levels at which a	
minor component can be detected. Perform mixture interpretation, as	
appropriate, to aid in determining the most likely major and minor genotypes.	
Run the remainder of the samples amplified for completion of the PowerPlex [®] Fusion 5C Amplification Training Checklist, varying injection times as necessary (or similar samples as assigned by trainer). Analyze the data and perform mixture interpretation as appropriate (basic major/minor interpretation).	

Biology/DNA Training Manual PowerPlex® Fusion 5C Capillary Electrophoresis and Data Interpretation Training Checklist

 \checkmark

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Practical Exercise/Discussions Continued Date Completed Process a minimum of two mock DNA batches (at least from amplification onward) and analyze each to the extent of batch review preparation. 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 analyzed in addition to mock cases as available. **Date Completed Quality Control** Perform critical reagents kit QC (PowerPlex® Fusion System Kit). Perform monthly maintenance/QC on 3130xL. Prepare and run a matrix/spectral calibration, as well as a spatial. Competency Date Completed Complete and pass written examination Complete and pass practical competency Successful completion of training and completency Supervised Cases Date Completed Perform capillary electrophoresis plate set up and loading of two batches (minimum of 32 samples each). Analyze results to extent of batch review preparation. Trainer Signatur Date Technical Leader Signature Date Statistics and Case Write Up Training Checklist Analyst: Primary Trainer Assigned: **Biology/DNA Training Manual**

Statistics and Case Write Up Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Page 65 of 89 Issu All printed copies are uncontrolled Technical Lead Plan Approval: _____

Comments:

Readings

Date Completed

Review relevant facilities, evidence, equipment, and documentation/reporting sections of the ISP Forensic Biology/DNA Quality Manual (current revision)

Review relevant DNA sections of the ISP Forensic Biology/DNA Casework Analytical Methods (current revision)

Applicable Validations/Performance Verifications, including cited Interature

Current version of STRMix User's Manual

Butler, J.M. Advanced Topics in Forensic DNA Typing. Interpretation. Academic Press (2015).

Evett, I.W. and Weir, B.S. Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists. Sinauer Associates, Inc. (1998).

Rudin, N., Inman, K. An Introduction to Forensic DNA Analysis, Second Edition. CRC Press (2002).

Bright, J.A. et al. The Effect of the Uncertainty in the Number of Contributors of Mixed DNA Profiles on Profile Interpretation. Forensic Science International: Genetics, 2014, 12: 208-214.

Hill, C.R. et al. U.S. Population Data for 29 Autosomal STR loci. Forensic Science International: Genetics, 2013; 7: e82-e83.

Crouse, C. et al. Analysis and interpretation of short tandem repeat microvariants and three banded allele patterns using multiple allele detection systems. Journal of Forensic Sciences, 1999; 44(1):87-94.

Biology/DNA Training Manual Statistics and Case Write Up Training Checklist

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Date Completed

Gill, P. et al. DNA Commission on the International Society of Forensic Genetics: Recommendation on the Interpretation of Mixtures. Forensic Science International, July 2006; 160(2): 90-101

Hares, D.R. Selection and Implementation of Expanded CODIS Core Loci in the United States. Forensic Science International: Genetics, 2015; 17: 33-34.

McEwen, J.E. Forensic DNA data banking by state crime laboratories. American Journal of Human Genetics, 1995; 56: 1487-1492.

Paoletti, D.R. et al. Empirical Analysis of the STR Profiles Resulting from Conceptual Mixtures. Journal of Forensic Sciences, 2005; 50(6):1-6.

Roby, R.K. et al. The National Institute of Justice's Expert Systems Testbed Project Proceedings of the 16th International Symposium on Human Identification, November 2005, Promega Corporation.

Taylor, D. Using Continuous DNA Interpretation Methods to Revisit Likelihood Ratio Behaviour. Forensic Science International: Genetics, 2014: 11, 144-153.

Taylor, D. et al. The Interpretation of Single Source and Mixed DNA Profiles. Forensic Science International: Genetics, 2013; 7(5): 516-528

Walsh, S.J., et.al. Comparing the Growth and Effectiveness of Forensic DNA Databases. Forensic Science International: Genetics, 2008; 1(1):667-668.

Weir, B.S. Matching and Partially-Matching DNA Profiles. Journal of Forensic Sciences, 2004; 49(5):1-6.

Lin, M.H. et al. The interpretation of mixed DNA profiles from a mother, father, and child trio. Forensic Science International: Genetics 44 (2020) 102175

Slooten, K. Likelihood ratio distributions and the (ir)relevance of error rates. Forensic Science International: Genetics 44 (2020) 102173

Biology/DNA Training Manual Statistics and Case Write Up Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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Practical Exercises/Discussions

Date Completed

Review at least 10 case files from a varie		
Observe case write up from at least 3 dif Minimum of 5 cases.	ferent analysts and a variety of case types.	
Perform statistical calculations for patern family samples (or similar samples as ass	nity on each of the previously extracted igned by trainer).	
Perform single source or mixture calcula appropriate, for one set of body fluid mix similar samples as assigned by trainer).	tions using the approved protocol, as xtures and the post-coital samples (or	
Perform mixture deconvolution and stati person mixtures amplified as part of the samples as assigned by trainer). The curr be used. Statistics will be calculated for k validated software program(s). Statistica scenarios to include different proposition appropriate.	istical calculations on the 2, 3, and 4 Amplification Training Checklist (or similar cent approved interpretation protocol will known contributors using the currently I calculations should cover a range of hs and conditioning profiles, as	
Attend courses/workshops on mixture ir validated software program(s) (as availal	aterpretation, statistics, and the currently ble).	
Write up a minimum of five mock DNA converted as would be done in the court analyzed (or known results) proficiency to mock cases. Note: Non-probative cases may be analyzed analyzed with the cases may be analyzed analyzed by the second seco		
Competency Complete and pass written examination		Date Completed
Complete and pass practical competency		
Testimony	aent	Date Completed
Biology/DNA Training Manual Statistics and Case Write Up	Revision 5 Issue Date: 01/06/2021	

Training Checklist

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Supervised Cases

Date Completed

Write up a minimum of 10 cases which include data analysis (I.e. No stop-at-quant only cases). Should include a variety of case types, at least 5 with statistics and 5 with CODIS entry (see CODIS Training Checklist).

Successful completion of training and competency:

Trainer Signature	Date
Technical Leader Signature	Date

Biology/DNA Training Manual Statistics and Case Write Up Training Checklist Revision 5 Issue Date: 01/06/2021 Page 69 of 89 Issuing Authority: Quality Manager

CODIS Training Checklist

Analyst:	
Primary Trainer Assigned:	
Technical Lead Plan Approval:	
Comments:	
Readings	Date Completed
NDIS Procedures Manual	
ISP CODIS Manual (current revision)	
Practical Exercises/Discussions	Date Completed
Enter at least 2 Specimens in STR Data Entry	
Locate the specimens entered through STR data entry and print specimen details reports for review by the CODIS Administrator.	
Perform staff search using Searcher	
CBT Trainings	
CODIS Lecture from CODIS Administrator	
Database only - Enter a minimum of two .cmf files using the import function.	
Perform a minimum of two keyboard staff searches.	

Biology/DNA Training Manual CODIS Training Checklist

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Competency	Date Completed
Complete and pass written examination	
NDIS Eligibility Exam	
Complete and pass practical competency To be completed in conjunction with the Statistics and Cas	se Write Up/Database Analyst Training
Testimony	Date Completed
Mock court/oral communication assessment Checklist:	
Supervised Cases	Date Completed
Write up at least 5 cases with CODIS entry	
Successful completion of training-and competency:	
Trainer Signature	Date
Technical Leader Signature	Date
Biology/DNA Training Manual CODIS Training Checklist Page 71 of 89 All printed copies are uncont	Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager rolled

PowerPlex® Y23 Training Checklist

Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:			
Comments:			
Readings			Date Completed
Review relevant facilities, evidence, sections of the ISP Forensic Biology	, equipment, and doc. DNA Quality Manual	imentation/reporting current revision)	
Review relevant DNA sections of th Methods (current revision)	e ISP Forensic Biology,	DNA Casework Analytical	
PowerPlex® Y23 Technical Manual			
ISP Casework PowerPlex® Y23 Valid	ation Report		
SWGDAM Y-STR Interpretation Guid	delines (2014)		
"Y-STR Analysis: PowerPlex [®] Y23 Sy Analytical Methods	stem" Section of ISPFS	S Biology/DNA Casework	
Moore, D. et al. Description of arter with excessive quantities of backgro International: Genetics 24 (2016) 44	facts in the PowerPlex ound female DNA. For 4–50.	Y23 [®] system associated ensic Science	
Biology/DNA Training Manual		Revision 5	
PowerPlex [®] Y23 Training		Issue Date: 01/06/2021	
Checklist	Page 72 of 89	Issuing Authority: Quali	ty Manager
Practical Exercises/Discussions		Date Completed	
--	--	----------------	
Lecture on YSTR's (to cover at minimum: background, an Թխճունուց ՆKrestuitsPerformance of the PowerPlex Y23® forensic genetic casework. Forensic Science Internations Ծանգութ տել 2011 թերթի գինել են ու	nalysis, statistics, and kit on trace samples in al: Genetics Supplement e batch of Y23 samples		
លិចនាមានលាលកាល់ពីឧត្យទៅនេះ Device ខែទាការទោងដៅមុខាភៅព្រះដែលដល់សានកា single multiplex Y-STR analysis system for casework and ក្រសាលន៍ ស្រីវីមិន ទោះ សារនៃជាការដែលខាត់ស្រីខាត់ទៅតែ ស្រីវីមិសិន អំពីស្រី ស្រីវីមិសិន អំពីស្រីខាត់ អំពីស្រីខាត់ ក្រសាលន៍ ស្រីវីមិន ស្រីវីមិសិន អំពីស្រីខាត់ស្រីខាត់ អំពីស្រីខាត់ អំពីស្រីខាត់ អំពីស្រីខាត់ អំពីស្រីខាត់ អំពីស្រ	ውærPlex Y23® System: A database samples. 5ቁs.		
Observe at least 2 YSTR case write ups, at least one case	e with statistics.		
Process provided practice samples (amplification, CE, ar statistical calculations)	nalysis, interpretation, and		
Competency		Date Completed	
Complete and pass written examination			
Complete and pass practical competency			
Testimony		Date Completed	
Mock court/oral communication assessment			
Supervised Cases		Date Completed	
Minimum of two batches (minimum of 5 cases each), at concentration/normalization	least one which includes		
Successful completion of training and competency:			
	_/		
Trainer Signature	Date		
Biology/DNA Training Manual PowerPlex® Y23 Training	Revision 5 Issue Date: 01/06/2021		

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Checklist

Technical Leader Signature

Date

Administrative/Technical Review Training Checklist - Serology

_____/ ____

Analyst:	
Primary Trainer Assigned:	
Technical Lead Plan Approval:	
Comments:	
Readings Practical Exercises/Discussions	Date Completed Date Completed
Read relevant review sections of ISP Forensic Biology Quality Manual (current Practice) serology technical reviews, minimum of 10 cases (to include both Y-screen and traditional screening cases)	
Competency	Date Completed
One serology case review	
Successful completion of training and competency:	
/ Trainer Signature Date	
//	
Biology/DNA Training ManualRevision 5Administrative/TechnicalIssue Date: 01/06/2021Review Training Checklist -Page 74 of 89SerologySerology	ity Manager

Technical Leader Signature

Date

Administrative/Technical and Batch Review Training Checklist -STRs

Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:	<u> </u>	-	
Comments:			
Readings			Date Completed
Reactical Exarcises Discussions of ISF	P Forensic Biology O	uality Magual (current	Date Completed
revision) Practice STR technical reviews mini	mum of 10 cases (to	include at least 5 STRMix	
cases).			
Practice STR batch review, minimum	n of 1 evidence and	1 reference	
Competency Complete and pass oral exam			Date Completed
One STR case review			
One batch review			
Successful completion of training an	d competency:		
· · · · · · · · · · · · · · · · · · ·		_/	
Trainer Signature		Date	
		/	
Technical Leader Signature		Date	
Biology/DNA Training Manual		Revision 5	
Administrative/Technical and		Issue Date: 01/06/20)21
Batch Review Training Checklist	Page 75 of 89	Issuing Authority: Qu	Jality Manager

Administrative/Technical and Batch Review Training Checklist -YSTRs

Analyst:			
Primary Trainer Assigned:			
Technical Lead Plan Approval:		_	
Comments:			
Readings			Date Completed
Read relevant review sections of ISP	Forensic Biology Qu	uality Manual (current	
revision) Practical Exercises/Discussions			Date Completed
Practice YSTR technical reviews, min with statistics).	imum of 10 cases (t	o include at least 3 cases	
Practice YSTR batch review, minimu	m of 1 batch		
Competency			Date Completed
Complete and pass oral exam			
One YSTR case review			
One YSTR batch review			
Successful completion of training and	d competency:		
Trainer Signature		_/ Date	
		1	
Technical Leader Signature		Date	
Biology/DNA Training Manual		Revision 5	
Administrative/Technical and Batch Review Training Checklist - YSTRs	Page 76 of 89	Issue Date: 01/06/2 Issuing Authority: O	021 Juality Manager

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Database Offender Sample Handling Training Checklist

Primary Trainer Assigned: _____

Technical Lead Plan Approval: _____

Comments:



Readings

Review relevant DNA Tracker sections of the ISP Forensic Biology Database Analytical Methods (current revision)

Kaye, J.A. Correspondence About Handling Evidence in cases of Acquired Immune Deficiency Syndrome (AIDS) [letter]. American Journal of Forensic Medicine and Pathology, March, 1986; 7(1):87-88. Practical Exercises/Discussions

Observe analyst(s) receiving/entering/storing offender samples and court orders, checking offenses, and using the DNA Tracker System.

Receiving/entering/storing offender samples and court orders, checking offenses, and using the DNA Tracker System, under direct observation of analyst. Minimum of 100 offender samples and/or court orders.

Lecture/Discussion over DNA Database Accessioning.

Competency

Date Completed

- Date Completed

Complete and pass written examination

Supervised Offender/Court Order Receipt, Offense Confirmation, and Tracker Entry. Minimum of 100 offender samples, minimum of 10 court orders.

Biology/DNA Training Manual Database Offender Sample Handling Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Page 77 of 89 Issu All printed copies are uncontrolled Successful completion of training and competency:



Biology/DNA Training Manual Database Offender Sample Handling Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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Database Analyst Training Checklist

Analyst: _____

Primary Trainer Assigned: _____

Readings Date Completed Review relevant facilities, evidence, equipment, and documentation/reporting sections of the ISP Forensic Biology/DNA Quality Manual (current revision).
Review relevant facilities, evidence, equipment, and documentation/reporting sections of the ISP Forensic Biology/DNA Quality Manual (current revision).
Review relevant DNA sections of the ISP Forensic DNA Database Analytical Methods (current revision). EZ1® Advanced User Manual. Applicable Validations/Performance Verifications, including cited literature. Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (FBI) (current revision) and associated guidance document.
EZ1® Advanced User Manual. Applicable Validations/Performance Verifications, including cited literature. Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (FBI) (current revision) and associated guidance document.
Applicable Validations/Performance Verifications, including cited literature. Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (FBI) (current revision) and associated guidance document.
Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (FBI) (current revision) and associated guidance document.
The Evaluation of Forensic DNA Evidence (National Research Council), National Academy Press, Washington, D.C. (1996).
DNA Technology in Forensic Science (National Research Council), National Academy Press, Washington, D.C. (1992).
Relevant Scientific Working Group on DNA Analysis Methods (SWGDAM) Guidelines Documents (current revisions). <u>https://www.swgdam.org/publications</u>
Butler, J.M. Fundamentals of Forensic DNA Typing. Academic Press (2009).
Biology/DNA Training Manual Revision 5
Database Analyst Training Issue Date: 01/06/2021
Checklist Page 79 of 89 Issuing Authority: Quality Manager

Butler, J.M. Advanced Topics in Forensic DNA Typing: Methodology. Academic Press (2011). Technical Lead Plan Approval: _____

Comments:



Biology/DNA Training Manual Database Analyst Training Checklist

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Readings Continued

Butler, J.M. Advanced Topics in Forensic DNA Typing: Interpretation. Academic Press (2011).

Plexor[®] HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems Technical Manual

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide

Graham, E.A.M. et al. Can Post-Mortem Blood Be Used for DNA Profiling After Peri-Mortem Blood Transfusion? International Journal of Legal Medicine, 2007; 121:18-23.

Horsman, K.M. et al. Forensic DNA Analysis on Microfluidic Devices: A Review. Journal of Forensic Sciences, 2007; 52(4): 784-798.

Jeffreys, A.J.; Wilson, V. and Thein, S.L. Individual-specific 'fingerprints' of Human DNA. Nature, December 12-18, 1985; 318(6046):577-579.

Montpetit, S.A. et al. A Simple Automated Instrument for DNA Extraction in Forensic Casework. Journal of Forensic Sciences, 2005; 50(3):1-9.

Krenke, B.E. et al. Developmental Validation of a Real-Time PCR Assay for the Simultaneous Quantification of Total Human and Male DNA. Forensic Science International: Genetics, December 2008; 3(1):14-21.

Valasek, M.A.; Repa, J.J. The Power of Real-Time PCR. Advances in Physiology Education, 2005; 29(3):151-159.

Swango, K.L. et al. A quantitative PCR Assay for the Assessment of DNA Degradation in Forensic Samples. Forensic Science International, April 2006; 158(1):14-26.

PowerPlex[®] Fusion System for Use on the Applied Biosystems[®] Genetic Analyzers Technical Manual

GeneAmp® PCR System 9700 User's Manual

PCR: The Polymerase Chain Reaction. BirkHauser Boston (1994). Ch 1, 3, and 21.

Biology/DNA Training Manual Database Analyst Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Date Completed

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Readings Continued

Date Completed

Budowle, B. et al. STR Primer Concordance Study. Forensic Science International, December 15, 2001; 124(1):47-54.

Edwards, M.C. and Gibbs, R.A. Multiplex PCR: advantages, development, and applications. PCR Meth. Appl. 1994; 3: S65-S75

Oostdik, K. et al. Developmental Validation of the PowerPlex[®] Fusion System for Analysis of Casework and Reference Samples: A24-Locus Multiplex for New Database Standards. Forensic Science International: Genetics, 2014; 12: 69-76.

Scherczinger, C.A. et al. A Systematic Analysis of PCR Contamination. Journal of Forensic Sciences, 1999; 44(5):1042-1045.

Walsh, P.S.; Erlich, H.A. and Higuchi, R. Preferential PCR amplification of alleles Mechanisms and solutions. PCR Meth. Appl. 1992; 1: 241-250.

PowerPlex[®] Fusion System for Use on the Applied Biosystems[®] Genetic Analyzer Technical Manual

Applied Biosystems® 3130/3130xl Genetic Analyzers Users Guide

Allor, C. et al. Identification and Characterization of Variant Alleles at CODIS STR Loci. Journal of Forensic Sciences, 2005; 50(5):1-6.

Budowle, B. et al. Population Data on the Thixteen CODIS Core Short Tandem Repeat Loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, and Trinidadians. Journal of Forensic Sciences, November, 1999; 44(6): 1277-1286

Buel, E. et. al. Capillary Electrophoresis STR Analysis: Comparison to Gel-Based Systems. Journal of Forensic Sciences, 1998; 43(1):164-170.

Butler, J.M. et al. Application of dual internal standards for precise sizing of polymerase chain reaction products using capillary electrophoresis. Electrophoresis, 1995, 16: 974-980.

Butler, J.M. Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing. Journal of Forensic Sciences, 2006; 5(2):253-265.

Biology/DNA Training Manual Database Analyst Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

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Readings Continued

Butler, J.M. New Resources for the Forensic Genetics Community Available on the NIST STRBase Website. Forensic Science International: Genetics, 2008; 1(1):97-99.

Chakraborty, R. et al. The utility of short tandem repeat loci beyond human identification: Implications for development of new DNA typing systems. Electrophoresis, 1999 20(8):1682-1696.

Fowler, J.C. et al. Repetitive Deoxyribonucleic Acid (DNA) and Human Genome Variation--a Concise Review relevant to Forensic Biology. Journal of Forensic Sciences, 1988; 33():1111-1126.

Mansfield, E.S. et al. Sensitivity, reproducibility, and accuracy in short tandem repeat genotyping using capillary array electrophoresis. Genome Research, 1996 6: 893-903.

Practical Exercises/Discussions

Trainer demonstration of associated worksheets.

Obtain a minimum of 5 buccal FTA, 2 buccal cotton swabs, 5 buccal foam swabs, and 2 bloodstain samples from different individuals (the same individuals can be used for each different sample type). Extract an appropriately sized cutting from each sample on the Qiagen BioRobot EZ1 using the appropriate protocol and a 200ul elution volume. Extract a second cutting from each buccal FTA sample using a 50ul elution volume. (These samples will be referred to as "extraction mock samples" for the exercises below).

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 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.

Biology/DNA Training Manual Database Analyst Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Page 83 of 89 Issu All printed copies are uncontrolled Date Completed

Date Completed

Practical Exercises/Discussions Continued

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.

Perform quantitation and normalization calculations on each of the extraction mock samples. Compare the concentration obtained from duplicate samples extracted with different elution volumes and different sample types.

Prepare a sensitivity 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. Ampl each using the approved protocol.

Amplify each of the extraction mock samples.

Obtain 46 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. Perform direct amplification using the approved protocol.

Obtain buccal FTA samples from a minimum of 24 different individuals (including the buccal FTA samples collected previously). Punch each sample onto a plate with the BSD puncher. Perform direct amplification on the plate using the approved protocol.

Perform Genetic Analyzer instrument setup (trap flush, water wash, buffer change, water change, polymer change or replenish, and array change if necessary. Trainer should review processes at minimum).

Run the sensitivity dilution series 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.

Biology/DNA Training Manual Database Analyst Training Checklist Revision 5 Issue Date: 01/06/2021 Issuing Authority: Quality Manager

Page 84 of 89 Issu All printed copies are uncontrolled Run all direct amp samples, varying injection times as necessary and analyze the data.

Practical Exercises/Discussions Continued

Process two full plates of previously-typed offender samples. 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.

Quality Control

Perform monthly maintenance/QC on EZ1.

Perform critical reagents kit QC Plexor® HY System Kit.

Perform monthly maintenance/QC on 7500.

Perform annual maintenance/QC on 7500.

Perform critical reagents kit QC PowerPlex® **Fusion** Sys ter

Perform annual maintenance/QC on 9700.

Perform monthly maintenance/QC on 3130xL

Prepare and run a matrix/spectral calibration, as well as a spatial.

Competency

Complete and pass written examination

Complete and pass practical competency

Note: Approval to perform supervised DNA database plates must be obtained from the Quality Manager and will be after completion of all other requirements.

Biology/DNA Training Manual Database Analyst Training Checklist

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Date Completed

Date Completed

Date Completed

/	
Trainer Signature	Date
Technical Leader Signature	Date
Supervised Cases	Date Completed
Complete two database sample sets, one of which is an extract	ed batch.

Successful completion of training and competency:

Biology/DNA Training Manual Database Analyst Training Checklist Revision 5 Issue Date: 01/06/2021 Page 86 of 89 Issuing Authority: Quality Manager

Technical Review for DNA Database Transing Ch	ecklist
Analyst:	
Practical Exercises/Discussions	Date Completed
Observe processing of at least one direct amplification plate	
Mock technical review of one direct amp plate	
Mock technical review of one EZ1 extracted sample set	
Competency Complete and pass oral exam	Date Completed
One technical review of a sample set (either direct amp or EZ1)	
Primary Trainer Assigned:	
Technical Lead Plan Approval	
Comments:	
Readings	Date Completed
Applicable sections of DNA Database Analytical Methods	

Biology/DNA Training ManualRevision 5Technical Review for DNAIssue Date: 01/06/2021Database Training ChecklistPage 87 of 89All printed copies are uncontrolled

Successful completion of training and competency:

	/		
Trainer Signature		Date	
	/_		
Technical Leader Signature		Date	
Supplies, Quality C	ontrol, and Rea	gents Training	Shecklist
Analyst:			•
Primary Trainer Assigned:			
Technical Lead Plan Approval:			
Comments:			
Readings			Date Completed
Review relevant facilities, evidence,	equipment, and docur	nentation/reporting	
Practical Exercises/Discussions	/DNA Quality Manual (d	current revision)	- Date Completed -
Review relevant section of the ISP F Observe analyst(s) preparing "in-ho (current revision)	orensic Services Health use" reagents and calls	and Safety Manual rating the pH meter.	
Observe analyst(s) ordering and rec SDS for reagents in Biology Section	eiving laboratory suppl	ies.	
Perform weekly quality control cher Approved chemicals/incompatibiliti	cks es list		
Perform non-instrumental monthly Approved supplies/services list	quality control checks.		
Perform non-instrumental quarterly P-card procedures	y quality control checks		
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