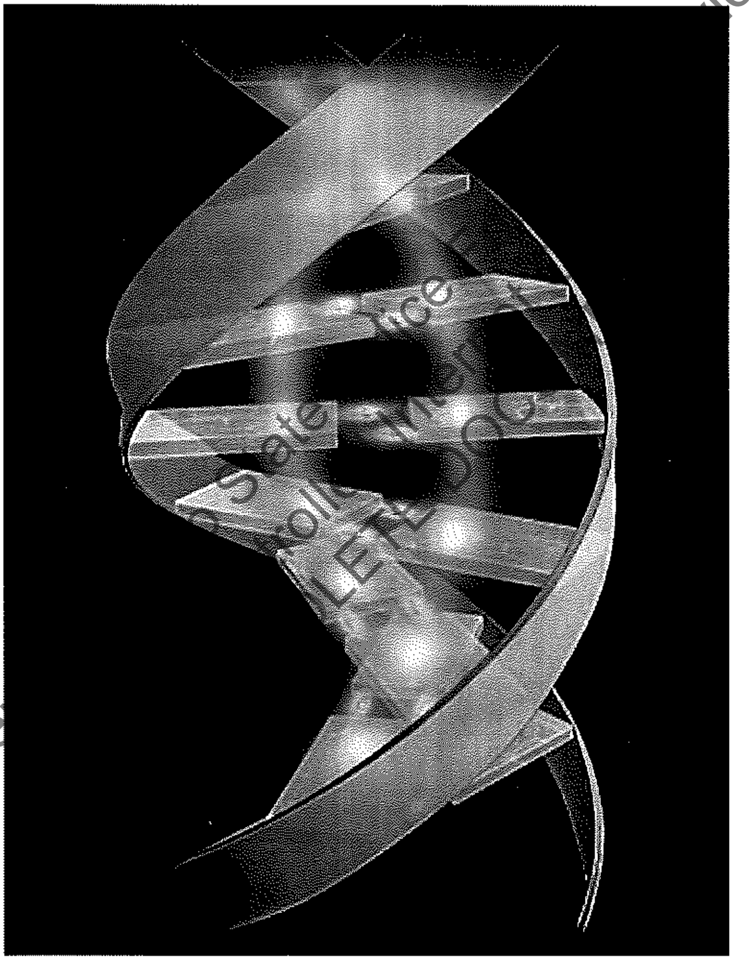
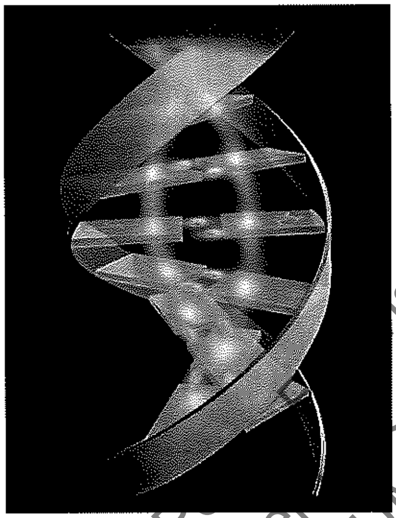
ISP FORENSIC BIOLOGY QUALITY /ANALYTICAL METHODS MANUAL



Forensic Biology Quality/Analytical Methods Manual

Revision #7



APPROVED FOR CASEWORK
February 4, 2008

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Forensic Biology Quality/Analytical Method Manual

REVISION RECORD

The following table must be filled out when revisions to the Biology Quality/Procedure Manual are made.

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

Date	Revision #	Description	Addition	Deletion	Initials
10/31/05	4	Added rtPCR SOPs/forms (BI-207, 206-BI, 209-BI), combined SOP's into 1 manual, clerical errors fixed.	X		CRH
11/30/05	N/A	BI-208 4.3.1.3 and BI-210 4.4.2.5 modified to only allow +3 amplification on CODIS samples			CRH
4/24/06	5	Added Mideo (BI-119) and EZ1 (BI-200 addition) SOP's and modified relevant forms, removed Quantiblot SOP's/forms (BI-202/206, 208/231/240/241/243/245/247-BI), changed Stimas SOP's to DNA Tracker (BI-301/302/303/310), updated security, clerical errors.	X	X	CRH
1/29/07	6	Added critical equipment inventory (401-QC), pH calibration record (403-QC), evidence vault QC (404B-QC), Quantifiler as critical reagent (419-QC), updated wording throughout, updated DNA testing policy, clerical errors.	X		CRH
2/4/08	7	Replaced GeneScan/GenoTyper with GeneMapper ID and added 3130 (BI-210), updated CODIS removal and DNA Tracker methods (BI-310 and BI-301), expanded mixture interpretation section, combined methods into 1 document, updated QA policies, added forms 216-BI, 218-BI and 422B-BI, clerical errors fixed throughout	X	X	CRH

Forensic Biology Quality/Analytical Method Manual
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20. Appendix B (Mixtures- minor component calculations)

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INTRODUCTION

The Forensic Biology Quality/Procedure Manual is not a public document. Copies of the manual, or portions thereof, will be released only to individuals having official business and upon proper discovery requests relating to a specific case(s).

1.0 STATEMENT OF PURPOSE AND OBJECTIVES

1.1 Statement of Purpose: ISP Forensic Biology exists to provide quality, unbiased and cost-effective analyses in the identification of biological substances and their source(s) relevant to the investigation and prosecution of criminal offenses in Idaho. The ISP Forensic Biology Quality/Procedure Manual, along with the ISP Forensic Services Quality/Procedure Manual, provide the framework for the evaluation of QC (Quality Control) measures utilized in Forensic Biology to achieve that purpose.

1.2 Objectives:

1.2.1 To develop and maintain, through annual review and revision (where necessary), a system of quality procedures, analytical methods, and controls to ensure quality up-to-date personnel training, biological screening and DNA analyses.

1.2.2 To evaluate (and revise where appropriate) through proficiency testing, audits, and other means of review, the thoroughness and effectiveness of biology personnel training, procedures and QC measures.

1.2.3 To remain scientifically neutral by basing case/evidence acceptance and analysis decisions, case reports and testimony solely on sound scientific rationale.

1.2.4 To develop and use practices that respect and protect the right of privacy for the genetic profiles developed in forensic casework or for database entry.

1.2.5 To provide high quality training, technical and informational assistance, biological analyses, written reports and testimony.

1.2.6 To provide all services in a cost-effective and timely manner.

2.0 ORGANIZATION AND MANAGEMENT

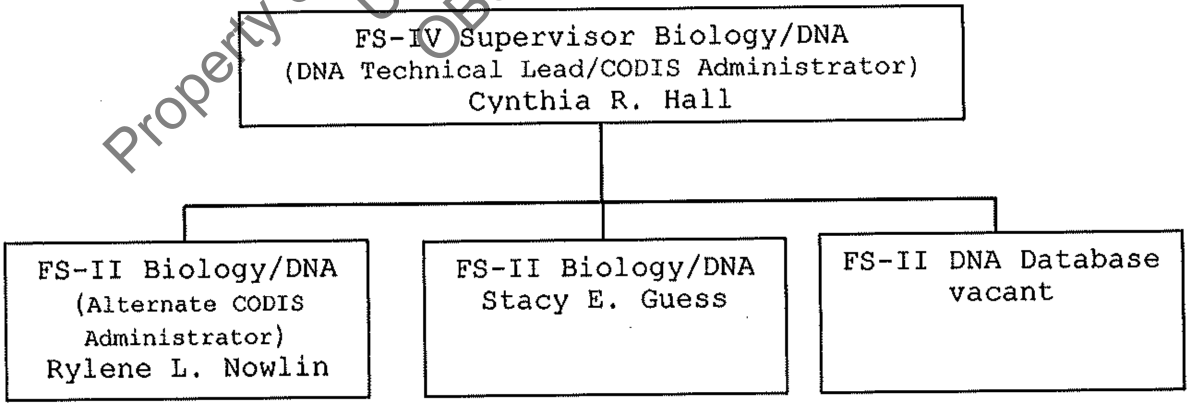
2.1 Organizational Chart and Functional Structure

2.1.1 An organizational chart for ISP Forensic Services appears in the ISP Forensic Services Quality/Procedure Manual. The Forensic Biology organization is delineated below.

2.1.2 An organizational chart for the Idaho State Police appears in the ISP Policy Manual.

2.2 Authority and Accountability in Forensic Biology

2.2.1 The Quality Assurance Standards for Forensic DNA Testing Laboratories and Convicted Offender DNA Databasing Laboratories, developed by the DAB, serve as a model for the ISP Forensic Biology QA Program. These standards delineate specific responsibilities and authority for the DNA Technical Manager and DNA CODIS Manager (see standard 4.1 of the FBI quality audit document). A copy of the document may be found in the ISP Forensic Biology Training Manual. Additionally, the ISP Forensic Services Quality/Procedure Manual designates specific authority for the DNA Technical Manager and DNA CODIS Manager.



Note: Changes (personnel) to this page do not require new revision numbers.

3.0 PERSONNEL QUALIFICATIONS AND TRAINING

3.1 Job Descriptions

General personnel qualifications and responsibilities, as well as personnel record retention policies, are described in the ISP Forensic Services Quality/Procedure Manual. Complete job descriptions are available through the Idaho Division of Human Resources web site: (http://dhr.idaho.gov/dhrapp/stateJobs/JobDescriptions.aspx).

3.2 Training

Refer to ISP Forensic Biology Training manual.

3.3 Qualifications

Education, training and experience for Forensic Biology personnel is formally established in the following minimum requirement specifications (Minimum requirements for individual positions may be reviewed at the time of job announcement and may exceed those delineated below). Periodic review of continuing education and overall performance is accomplished during the annual employee evaluation. Opportunities are provided by an FS training budget.

3.3.1 Forensic Biology/DNA Supervisor/Technical Manager

It is assumed for the purposes of this document (and is currently the case), that in a laboratory system of the size of Idaho's, these functions will be served by a single individual.

3.3.1.1 Education

Must have at minimum, a Master of Science degree in a biological science. Successful completion of a minimum of 12 credit hours, including a combination of graduate and undergraduate coursework in genetics, biochemistry, molecular biology and statistics (or population genetics).

3.3.1.2 Training

Training and experience in molecular biology and DNA-based analyses from academic, governmental, private forensic and/or research laboratory(ies). Must also complete the FBI sponsored DNA auditor training within 1 year of appointment, if not already completed (dependant on FBI scheduling).

3.3.1.3 Experience

Must have a minimum of three years forensic human DNA laboratory experience as an analyst.

3.3.1.4 Continuing Education

Must stay abreast of developments relevant to forensic DNA analyses through the reading of current scientific literature and attendance (and participation) at DNA related seminars, courses and/or professional meetings, for a minimum of 8 hours per calendar year.

3.3.2 CODIS Manager

This function may or may not be served by the Forensic Biology/DNA Supervisor. It is assumed for the purposes of this document (and is currently the case) that in a laboratory system of the size of Idaho's, the functions of casework and database CODIS Managers will be served by a single individual. An Alternate CODIS Manager will also be appointed and must meet the same qualifications as the CODIS Manager.

3.3.2.1 Education

Must have at minimum, a Bachelor of Science degree in a biological science and successfully completed college coursework in genetics, biochemistry, and molecular biology. Must also have completed coursework and/or training in statistics (or population genetics).

3.3.2.2 Training

A combination of training and experience in the use of computers, and database systems in a laboratory/scientific setting. Must also complete the FBI's CODIS software training and the DNA auditor training within six months of appointment if not already completed (dependant on FBI scheduling).

3.3.2.3 Experience

Must possess a working knowledge of computers, computer networks, computer database management and have an understanding of DNA profile interpretation for database and casework functions, to include mixture interpretation. Must be or have been a qualified DNA analyst.

3.3.2.4 Continuing Education

Must stay abreast of developments relevant to CODIS/NDIS database management, computer and data security and computer networks through the reading of appropriate literature and attendance (personal or that of the Alternate CODIS Manager) at the bi-annual CODIS State Administrators' meetings and annual CODIS conference. Further educational development to be obtained through relevant DNA related courses and/or seminars, for a minimum of 8 hours per calendar year.

3.3.3 DNA Analyst

The following delineate requirements for a DNA casework or database analyst whose responsibilities include performing genetic analyses on the capillary electrophoresis instruments and data interpretation. DNA extraction, quantification, and amplification set-up may be performed by appropriately trained laboratory technicians and/or those performing the biological screening of evidence following task-specific training and successful completion of a qualifying examination.

3.3.3.1 Education

Must have at minimum, a Bachelor of Science degree in a biological science and successfully completed college coursework in genetics, biochemistry, and molecular biology. Must also have completed coursework and/or training in statistics (or population genetics).

3.3.3.2 Training

Training in DNA analyses through academic, governmental, private forensic and/or research laboratory(ies). If received elsewhere, documented training must meet or exceed that outlined in the ISP Forensic Biology training manual. Must successfully complete a qualifying examination prior to performing analyses on database or forensic casework samples.

3.3.3.3 Experience

Must have a minimum of six months forensic human DNA laboratory experience.

3.3.3.4 Continuing Education

Must stay abreast of developments relevant to forensic DNA analyses through the reading of current scientific literature and attendance (and participation) at DNA related seminars, courses and/or professional meetings, for a minimum of 8 hours per calendar year.

3.3.4 Forensic Biologist

The following delineate requirements for those individuals responsible for the screening of evidence for the presence of biological substances and reporting and giving testimony regarding their findings.

3.3.4.1 Education

Must have a Bachelor of Science in a biological science.

3.3.4.2 Training

Training specific to this job function in a governmental and/or private forensic laboratory. If received elsewhere, documented training must meet or exceed that outlined in the ISP Forensic Biology training manual. Must successfully complete a qualifying examination prior to performing forensic casework.

3.3.4.3 Experience

Prior to participating in independent forensic casework, must have a minimum of six months forensic laboratory experience in the area of biological screening and/or DNA analysis.

3.3.4.4 Continuing Education

Must stay abreast of relevant developments through the reading of current scientific literature and attendance (and participation) at seminars, courses and/or professional meetings.

3.3.5 Biology Laboratory Technician

3.3.5.1 Education

Minimum of two years of college to include scientific coursework (lecture and lab); Bachelor of Science degree in a biological science is preferred.

3.3.5.2 Training

Must receive on the job training specific to assigned duties and successfully complete a qualifying examination before participating in forensic DNA typing or forensic casework responsibilities.

3.3.5.3 Experience

Prior to participating in any forensic DNA typing responsibilities or forensic case processing activities, technician must have a minimum of six months forensic laboratory experience in the area of Biology/DNA; one year is preferred.

3.3.5.4 Continuing Education

Must stay abreast of relevant developments through the reading of current scientific literature and attendance (and participation) at seminars, courses and/or professional meetings.

.0 FACILITIES

4.1 Laboratory Security

Security of the Forensic Services Laboratory is covered in the ISP Forensic Services Quality/Procedure Manual.

4.1.1 Forensic Biology Security

When not under the direct control of Forensic Biology personnel, evidence and in-progress work product will be secured either by closing and locking the Forensic Biology door or by its return to secure storage (one of the locked evidence refrigerators/freezers/file cabinets or the analyst's personal evidence cabinet). Only Forensic Biology Personnel will have access to the locked storage and laboratory areas. Persons outside the Forensic Biology unit will not be allowed access to the Forensic Biology laboratories. Exceptions will be made in case of emergencies, for maintenance, safety, and/or equipment service needs, and for required annual quality and DNA audits. At these times, access will be limited to only required individuals, the individual(s) will be accompanied by biology program personnel, and all evidence will be placed in secured storage for the duration of the individual(s) being present in the laboratory.

4.1.2 CODIS Security

The CODIS workstation is located in the locked CODIS office and the CODIS Server is located in the secured server room in the CJIS Section. The following security measures have been implemented:

4.1.2.1 Only Forensic Biology personnel will have access to the CODIS office. When a biology staff member is not present, the office will be secured by closing and locking the door.

4.1.2.2 Only the CODIS State Administrator, designated Forensic Biology staff and CJIS personnel will have access to the CODIS Server.

4.1.2.3 A differential backup of the CODIS server will be performed each weekday. A full backup will be performed once weekly with the backup tape being stored off-site. At any given time, one month of data will be stored offsite.

4.1.2.4 Only Forensic Biology Personnel that have gone through the NDIS application and approval process will have user-names and passwords for CODIS.

4.1.2.5 CODIS users must log in each time they use CODIS and log out prior to leaving the CODIS Workstation.

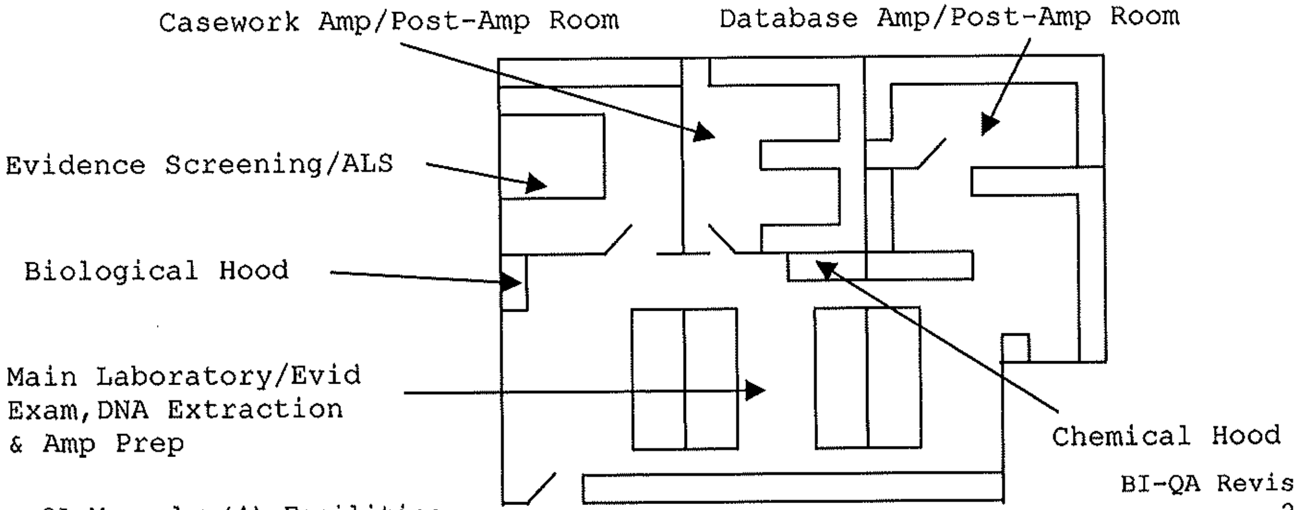
4.1.2.6 DNA Tracker, the convicted offender sample-tracking database resides on the ISP intranet and is accessible, only to personnel designated by the Biology/DNA Supervisor.

4.1.2.7 Personal and identifying information on convicted offenders (hard and electronic/DNA Tracker copies) are stored separately from the DNA profile (CODIS) obtained. The DNA profiles are directly associated <u>only</u> with a unique Idaho Convicted Offender ID number, assigned by DNA Tracker upon sample entry.

4.1.2.8 CODIS sample information is released only in accordance with 19-5514 of the Idaho DNA Database Act of 1996, the Privacy Act Notice in Appendix E of NDIS procedures, and the FBI/CODIS Memorandum of Understanding.

4.2 Forensic Biology Laboratory Set-up

The Forensic Biology Laboratory is designed to minimize contamination potential during the processing and analysis of forensic and convicted offender samples. The diagram below depicts the laboratory set-up and delineates the separate areas for evidence examination, DNA extraction, PCR Amplification Set-up and Amplified DNA processing and storage. Some steps of the pre-amplification processes may be conducted in the same area of the main laboratory; however, these steps are separated by time.



4.3 Laboratory Cleaning and Decontamination

In order to minimize the potential for sample contamination, careful cleaning of laboratory work areas and equipment must be conducted on a routine basis. The efficacy of the procedures used is monitored through the use of controls within the analysis process (see the interpretation guidelines section in BI-210). It is also important that each analyst use proper 'clean technique' at all times when in the laboratory, which includes but is not limited to, using only disposable barrier pipette tips and autoclaved microcentrifuge tubes, using a tube de-capping tool, and wearing gloves, a labcoat, and masks as appropriate. Additionally, biology personnel will be required to wear laboratory scrubs and dedicated shoes while in the laboratory.

4.3.1 All working **benchtop** surfaces will be cleaned with 10% bleach or Dispatch solution before and after use and as part of the monthly QC procedure. Clean white paper and/or a KayDry will be placed on the workbench prior to use and changed as appropriate and necessary.

4.3.2 All **small tools/instruments** (i.e. forceps, scissors, etc.) will be cleaned/rinsed with ethanol or germicidal instrument cleaner prior to use and between samples. Kimwipes, used to dry the instrument after cleaning/rinsing, will be single use only.

4.3.3 **Pipettes** are to be cleaned thoroughly with Dispatch solution as part of the monthly QC procedure and anytime the barrel comes in contact with DNA or any biological fluid.

4.3.4 All **centrifuges** are to be wiped down (interior and exterior) with Dispatch solution as part of the monthly QC procedure and in the event of a spill.

4.3.5 The **thermal cyclers**, to include the heating block and exterior surfaces, are to be wiped down with ethanol or Dispatch solution as part of the monthly QC procedure. Individual wells should be cleaned as needed.

4.3.6 All work surfaces in the **amplification/post-amp rooms** are to be cleaned with 10% bleach or Dispatch solution before and after analysis and as part of the monthly QC procedure. Clean white paper and/or a KayDry is to be placed on the benchtop prior to use. Additionally, as part of the monthly QC procedure, the following are to be conducted:

the exterior surfaces of the genetic analyzers and real-time instruments wiped down with ethanol or Dispatch solution, top of the refrigerator/freezers and surface underneath each genetic analyzer wiped down/dusted, and floor mopped.

.0 EVIDENCE CONTROL

Evidence, Individual Characteristic Database (Convicted Offender) samples, and in progress work product, that is collected, received, handled, sampled, analyzed and/or stored by ISP Forensic Services is done so in a manner to preserve its identity, integrity, condition and security.

5.1 Laboratory Evidence Control

Procedures detailing evidence handling are contained in the ISP Forensic Services Quality/Procedure Manual. Portions of individual evidence items that are carried through the analysis process (i.e. substrate cuttings, extracts, amplified product and/or portions thereof) are considered work product while in the process of analysis and do not require sealing. Work product will be identified by labeling the individual sample tube with a unique identifier, or documenting the locations of individual samples within a plate of samples.

5.2 Forensic Biology Evidence Control/Sample Retention

5.2.1 DNA Packet

It has become increasingly important to retain evidence for possible future analyses and to secure samples for non-probative casework analyses that are necessary for the validation of any new technology. Therefore, a DNA packet is created for cases submitted for analysis to Forensic Biology, in which reference sample(s) are present, and/or positive Biological screening results are obtained (See BI-102). Any remaining DNA extracts, upon completion of analysis, will be placed into a sealed container (such as a plastic zip bag) and stored in the DNA packet.

5.2.2 Limited Sample

In every case, care should be taken to save ~1/2 of a sample for independent testing. If testing would consume all or nearly all of a sample **and** there is an identified suspect charged in the case, the accused must receive appropriate notification. Written and/or verbal notification will be given to the prosecuting attorney informing him/her of possible consumption and requesting defense counsel be notified of the situation. Before testing will commence, an allowance will be made for testing by another accredited laboratory agreed upon by both parties. Additionally, a letter from the prosecuting attorney must be received by the laboratory indicating whether or not the sample may be consumed.

5.2.3 Amplified Product

Amplified DNA product will not be retained after 1) the report has been issued in the case or 2) review of the offender sample data has been completed and certified for CODIS entry. In cases where both the evidence and associated DNA extract have been consumed, the amplified product will be retained in a sealed container within the product room freezer.

6.0 VALIDATION

Procedures for the validation of methods used in ISP Forensic Services are outlined in the ISP Forensic Services Quality/Procedure Manual. Validation data, results and summaries for those methods employed in Forensic Biology will be maintained in that section.

7.0 CHEMICALS/REAGENTS

General laboratory policies and procedures regarding the purchase of chemicals and preparation of reagents are covered in the ISP Forensic Services Quality/Procedure Manual.

7.1 COMMERCIALLY PURCHASED CHEMICALS

7.1.1 Biology Personnel should consult the electronic Chemical Inventory Log (Form 400-QC) prior to ordering. Chemical grade requirements should be checked and ordered as appropriate. The date ordered should be reflected in the log to avoid duplicate orders. An entry for chemicals not currently on the inventory will be made at this time to reflect the chemical, source, and order date. This inventory will be audited annually, at a minimum, and a printout placed in the Forensic Biology Reagent Binder.

Note: An order form/document must be filled out and approved by the section supervisor (indicated by date and initials) prior to placing the order.

7.1.2 Upon receipt of a chemical or reagent, the Chemical Inventory Log will be updated to reflect the new lot number, received date, quantity received, and quantity in stock. The order date will be removed at this time. The chemical(s) will be marked with the date received and the individual's initials, as well as any necessary hazard labels. If it is an outer container that the chemical/kit remains in until use, the inner container will be labeled with this information when removed for use. Packing slips should be checked to ensure appropriate accounting, including proper reagent grade, where applicable (this will be indicated by dating and initialing the packing slip and making notations as necessary). The packing slip and corresponding order document will be retained in the biology section. If an MSDS sheet came with the chemical, the MSDS binder should be checked for the presence of an MSDS sheet for that chemical. If one exists, no additional copy is kept; however, if a newer version is received, the old one should be replaced. If one does not exist, place one in the binder. For chemicals without MSDS, consult the manufacturer or one of the following websites for information:

http://www.hazard.com/msds
http://www.msds.com
http://www.ilpi.com/msds/

Note: Critical Reagents listed in 7.3 will be tracked on the individual QC forms, rather than the chemical inventory log.

7.1.3 Expired chemicals will be disposed of in an appropriate manner.

7.2 REAGENTS PREPARED IN-HOUSE

7.2.1 All biology reagents will be made with great care, following all quality and safety procedures. A mask will be worn by analysts during reagent preparation to help avoid the potential for contamination.

7.2.2 Each reagent has a corresponding form that provides instructions for how to make and store the reagent as well as a format to document the making of the reagent and components used. This form must be filled out. A reagent label must be made that has the lab lot number, the date, and the individual's/preparer's initials. The NFPA designation will be completed on all labels (see reagent sheets). Although the reagent is identifiable to lab personnel by lot number (which consists of the first few letters of the reagent name followed by the date in the form 'MMDDYY'), the reagent label should still bear the name of the reagent as well. Refillable squirt-bottles of water or ethanol will be labeled but need not bear dates or initials.

7.3 CRITICAL REAGENTS

CRITICAL REAGENTS are those reagents that, if improperly functioning, could result in significant loss or destruction of DNA and are not amenable (or it's not practical) to testing immediately before (e.g., use on forensic samples) each use. The reagents listed below have been identified as critical in Forensic Biology/DNA. These reagents must undergo a **QC ASSAY BEFORE** use on forensic casework and/or Convicted Offender samples. Reagents received at a later date but having the same lot number as those previously tested and determined acceptable need not have a QC check performed. Critical Reagents (in addition to other DNA-related reagents with manufacturer expiration dates) may be used beyond the listed expiration date for training purposes without any further testing, so long as expected results are obtained for all associated controls. The reagent must be labeled 'for training only' if it is to be retained once the expiration date has been reached.

ABACARD® HEMATRACE® TEST KIT (Form 410-QC)

OneStep ABACARD® p30 TEST KIT (Form 412-QC)

Quantifiler® Human DNA Quantification Kit (Form 419-QC)

STR Kit (Taq Polymerase checked with kits; Form 420-QC)

8.0 EQUIPMENT CALIBRATION AND MAINTENANCE

General laboratory procedures for the calibration and maintenance of equipment are covered in the ISP Forensic Services Quality/Procedure Manual.

8.1 BIOLOGY EQUIPMENT/INSTRUMENTATION

8.1.1 Analytical equipment significant to the results of examination and requiring routine calibration and/or performance verification will be listed on the BIOLOGY CRITICAL EQUIPMENT INVENTORY Spreadsheet (Form 401-QC). Information on the spreadsheet includes (as known or appropriate): equipment identity and its software, manufacturer's name, model, property number, serial number and/or unique identifier, and location. The inventory spreadsheet will be maintained in the Instrument QC binder or Biology QC binder as appropriate.

8.1.2 OPERATING MANUALS for most equipment/instrumentation will be maintained in the product information file (Manuals for the ABI PRISM™ 310 and 3130 Genetic Analyzers, ABI Prism™ 7000 Sequence Detection System, Thermal Cycler Verification Kit, and Thermal Cyclers will be maintained in the Amp/PostAmp Room in close proximity to the instruments). Exceptions may be made for manuals referred to for instructions. In these cases, the manual will be maintained in close proximity to the instrument.

8.1.3 MAINTENANCE/REPAIR/CALIBRATION LOGS will be maintained as follows:

The records for the ABI PRISM™ 310 and 3130 Genetic Analyzers, ABI Prism™ 7000 Sequence Detection System, and Thermal Cyclers will be maintained in the instrument QC binder.

Any equipment/instrumentation function (not documented on weekly, monthly, quarterly, or annual QC Check forms) will be recorded on the Equipment Maintenance/Repair form (Form 402-QC). Equipment Failure will also be reported on this form. This form and the QC check forms will be maintained in the Biology QC Binder, except as listed above.

8.1.4 EQUIPMENT FAILURE will result in that equipment being 'taken out of service'; an 'out of service' sign will be placed on the equipment and it will not be returned to service until it has passed appropriate performance testing. Actions are reported on Form 402-QC.

8.1.5 The SCHEDULE of QC Checks for both critical and non-critical equipment is as follows:

WEEKLY (Form 404A/B-QC)
(once per week with an interval between dates not less than 3 days and not exceeding 10 days)

- Nanopure System Check
- Refrigerator/Freezer Temperature Check
- Heating Block(s) Temperature Check
- Oven Temperature Check

MONTHLY (Form 406A/B-QC)
(once per month with an interval between dates not less than 15 days and not exceeding 45 days)

- Pipettes Cleaned
- Centrifuges Cleaned
- Lab Cleaned
- Autoclave Clean and Check Sterilization
- ABI 7000 Background Assay, Contamination Test, and Bulb Check
- BioRobot EZ1 grease O-rings
- 3130 Water Wash
- 3130 Water Trap Flush
- 310 and 3130 (C and E drives) computer defragmentation

QUARTERLY
(once per quarter with an interval between dates not less than 30 days and not exceeding 120 days) Note: * denotes critical equipment

- Thermal Cycler* Verification Tests (Form 408A-QC)
- Chemical Shower Check (Form 408B-QC)
- Eye Wash Station Check (Form 408B-QC)

ANNUALLY (Form 402-QC)
(once per calendar year with an interval between dates not less than 6 months and not exceeding 18 months) Note: * denotes critical equipment

- Pipette* Calibration/Performance Verification Check (outside vendor)
- Thermometers (outside vendor)
- Thermal Cycler Verification Kit* Calibration Check (outside vendor)
- Biological and Chemical Hoods Test (outside vendor)
- Digital Temperature Recording Devices Calibration Check (outside vendor)
- ABI PRISM™ 310* Genetic Analyzer Preventative Maintenance (outside vendor)
- ABI PRISM™ 3130* Genetic Analyzer Preventative Maintenance (outside vendor)
- ABI PRISM™ 7000* Sequence Detection System Preventative Maintenance(outside vendor)
- ABI PRISM™ 7000* Pure Dye Calibration and Regions of Interest (ROI's) verification (see 7000 User Guide for procedures/may be part of PM by request)
- Qiagen BioRobot EZ1 Preventative Maintenance (outside vendor)
- Microscope Cleaning/Preventative Maintenance (outside vendor)
- Centrifuge Calibration Check (outside vendor)
- Balance* Calibration Check (outside vendor)

In addition to the above schedule, personnel should check appropriate parameter function on all instrumentation with each use (including calibration of the pH meter at the time of use; documented on Form 403-QC), and run a matrix for the ABI PRISM™ 310 Genetic Analyzers and a spatial and spectral calibration for the ABI PRISM™ 3130 Genetic Analyzers as needed or following CCD camera and/or laser replacement/adjustment. Additionally, following the annual preventative maintenance, a sensitivity panel should be run on the 310 and 3130 and included in the QC binder as a verification of performance. Any problems noted should be brought to the attention of the necessary supervisory personnel and documented on Form 402-QC. Data for each new matrix will be filed in the instrument QC binder (see BI-210).

9.0 PROFICIENCY TESTING

General laboratory guidelines and practices for proficiency testing and retention are outlined in the ISP Forensic Services Quality/Procedure Manual. Additional Biology/DNA requirements are delineated below.

9.1 External DNA Proficiency Test Requirement. DNA analysts will participate in external proficiency tests, twice in every calendar year, in accordance with NDIS Procedures and the results reported to NDIS as necessary.

9.2 Inconclusive/Uninterpretable Proficiency Test Results. Typically, sample size/quantity in PCR DNA Proficiency Tests is sufficient for multiple analyses to be performed. Therefore, results of DNA proficiency tests are not likely to be either inconclusive, or uninterpretable (e.g., not meeting minimal rfu and/or statistical threshold for inclusion/exclusion). However, in the event data obtained in a proficiency test does not meet the standard guidelines for interpretation/conclusion, it will first be determined, by re-testing and communication with the vendor, that this is not an issue with a given sample(s). Once that determination has been made, the analyst obtaining the inconclusive data will be removed from casework/CODIS sample analysis until satisfactory completion of a competency test and review of the analyst's casework/CODIS analysis performed since the last successful proficiency test.

10.0 CORRECTIVE ACTION

Laboratory corrective-action and retention procedures are detailed in the ISP Forensic Services Quality/Procedure Manual.

11.0 FILE DOCUMENTATION AND REPORTS

Meticulous documentation is an important aspect of forensic work. In casework, the scientist's knowledge of case circumstance (and therefore their ability to discern potential significance) may be limited. It is also common to be called upon to testify months, or even years, after processing evidence for a given case. Careful observation and detailed note-taking will not only refresh the scientist's memory and provide support for the conclusion in the laboratory report, but might also provide additional information not thought to have been important at the time of evidence processing. General laboratory policies regarding case record and retention are described in the ISP Forensic Services Quality/Procedure Manual.

11.1 CASE NOTES

11.1.1 Each page of case notes should have the following: Laboratory Case Number, Date, Scientist's Initials and page number (in a form indicating page/total pages).

11.1.2 Case notes are associated with a particular report. Case notes for additional submissions (i.e., for supplemental reports) will be reflected in the page numbering as well.

11.1.3 All evidence submitted for biological screening should be transferred to the scientist (i.e., documented on the chain of custody) and bear the scientist's initials. This is the case regardless of whether or not they analyze the item of evidence (exception may be made in cases where communication with investigator/attorney identified select items of those submitted). A description of the evidence (e.g., packaging and what it is said to contain) should also appear in the case notes with a notation about not being examined at the time, if that's the case. Those items should also appear in the "not examined" statement of the report.

11.1.4 The description of evidence packaging should include the type and condition of seal(s). Differences in the description on a package versus ETS entry and/or accompanying submission form (or what the evidence is once opened) should be noted.

11.1.5 Whenever feasible, every attempt should be made to gain entry into the evidence without breaking the original seals. Any seal altered or created by a scientist will bear their initials and date across the seal.

11.1.6 Evidence descriptions should be "unique" inasmuch as possible (i.e., one pair blue jeans is **NOT** adequate). They should include, as appropriate and necessary for identification, colors, sizes (measurements where appropriate- e.g., knife and blade), manufacturer, model, brand, serial numbers or other identifiers and condition (e.g., worn, clean, torn, mud-caked, blood-soaked, etc.).

11.1.7 Photography, digital or otherwise, is often useful in documenting the appearance of evidence items. However, it is not meant to completely replace drawing, but instead as a supplement or in cases when drawing may be too difficult to accurately depict the item. Careful drawing and description result in careful and detailed examinations and, in many instances, may be a better choice than photography. Digital photographs will be transferred to, printed as necessary for case notes, and stored within the Mideo System; refer to BI-119 for Mideo instructions.

11.1.8 Evidence numbering must be unique for the purpose of possible later CODIS entry. Items should be numbered as follows (or other similar system):

A single item (e.g., a baseball cap; Item 57) for which:

≤ 1 area tested positive for a biological substance ≡ Item 57

≥2 areas tested positive for a biological substance(s) (in this instance 3 areas) ≡ Item 57-1, Item 57-2 and Item 57-3, or 57-A, 57-B and 57-C.

An item with multiple sub-items (e.g., a SAECK; Item 1)

≡ Item 1A, Item 1B, Item 1C, etc., the scientist should begin with the most relevant item if possible. Multiple areas ≡ Item 1A-1, Item 1A-2 etc.

11.1.9 The Biology Screening Case Summary Form (Form 101-BI) may be used for summarizing analyses if the scientist chooses.

11.1.10 If a form is used for more than one case, a copy of the 'completed' form should be made for any additional case files. Each copy should contain a reference regarding the location (case file) of the original document. For each file, the associated case should be listed and case data highlighted. In general, biology subfolders should be organized from front to back as follows: restitution where applicable, report, chronological case notes/forms, SAECK form where applicable, case review forms where applicable, copy of evidence submission form or ETS property form, phone/info log ('tangerine' paper may be used for ease of identification), followed by agency materials submitted with evidence. When the report has been issued, this documentation should be bound (e.g., stapled) together.

11.2 REPORTS

In the interest of consistency and clarity of reports between individual scientists the following format should be adhered to:

11.2.1 The report will contain the title Forensic Biology Report for biology screening reports, or Forensic DNA Report for DNA reports.

11.2.2 For clarity, when a statement(s) is about a particular Item (or multiple items listed individually), the "I" will be capitalized as in a name. When writing in general terms (i.e., the following items:) the "i" will remain lowercase.

11.2.3 The case submission information will include, at a minimum: case#, report date, case agency, agency case#, principals (victim, suspect, etc.), and offense date.

11.2.4 The body of the report will be separated from the case submission information by the following headings in the format below:

<u>RESULTS OF EXAMINATION</u>

Statements (see below) regarding evidence exam, results and conclusions. The order of statements should be, inasmuch as possible: 1) ositive statements (detection of body fluid), 2) inconclusive statements,

3) negative statements and 4) statements regarding (i.e. a list of) items not examined.

Disposition of Evidence

Statements (See below) regarding evidence retention and return.

Evidence Description

The following items were received in the laboratory via Federal Express (UPS, US Mail, etc.) on Month day, year. (or) The following items were received in the laboratory from Agency Representative (Agency) on Month day, year.

Description of items submitted for examination.

In the first report, all items should be listed (any items scientist took possession of, including reference samples). In supplemental reports, only those items relevant to the additional examinations need to be listed.

DNA reports, in which a DNA packet is checked out for analysis, will state: A tape sealed DNA packet envelope, created in the laboratory on Month day, year, and containing the following items:

Description of items contained within the DNA packet.

This report does or may contain opinions and/or interpretations, of the undersigned analyst, based on scientific data. The analyst's signature certifies that all of the above are true and accurate. *(Note: the interpretations statement does not need to be included in reports where all items submitted are being returned without analysis, or other instances when no conclusions or interpretations are made.)*

Signature

Name of Scientist
Title of Scientist

11.2.5 The following results/conclusions statements are to be used in a **biology screening report**, as dictated by the analysis findings (Where appropriate, descriptions, quantity, and/or locations of individual stains may be included in the corresponding statements. Portions of individual statements may be combined as needed.):

Semen Results/Conclusions Statements:

Chemical and microscopic analyses for the detection of semen were conducted on (items). Semen was confirmed by the presence of spermatozoa on (items). (or) Semen was not detected on (items). (or) No identifiable spermatozoa were detected on (items).

Chemical and microscopic analyses for the detection of semen were conducted on (items). Semen was confirmed on (items) by the presence of a single spermatozoon (or limited number of spermatozoa), which is (or may be) insufficient for further testing at this time.

Chemical, microscopic, and serological analyses for the detection of semen were conducted on (items). Semen was detected on (items) by the presence of the semen specific protein, p30; however, no spermatozoa were observed, which is insufficient for further testing at this time.

Results from presumptive chemical tests for the presence of semen were negative on (items).

Blood Results/Conclusion Statements:

Results from chemical and serological tests performed on (items) indicated the presence of human (or non-human) blood.

Results from presumptive chemical tests performed on (items) indicated the presence of blood; however, serological tests to determine the species of origin were not performed (or were inconclusive).

Results from presumptive chemical tests for the presence of blood were negative on (items).

Saliva Results/Conclusions Statements:

Results from chemical tests performed on (items) indicated the presence of an elevated level of amylase, an enzymatic component of saliva.

Results from chemical tests performed on (items) indicated (or did not indicate, or were inconclusive for) the presence of amylase, an enzymatic component of saliva.

Urine Results/Conclusions Statements:

Results from presumptive chemical tests performed on (items) indicated (or did not indicate, or were inconclusive for) the presence of urine.

Feces Results/Conclusions Statements:

Results from presumptive chemical tests performed on (items) indicated (or did not indicate, or were inconclusive for) the presence of feces.

Further Testing Statements (to be included at the end of the Results of Examination Section):

If additional testing is desired, please contact the laboratory.

DNA testing can be performed (or may be attempted) upon request and submission of a known blood sample(s) from [list name(s)]. Please contact the laboratory regarding the analysis request.

11.2.6 The following results/conclusions statements are to be used in an **STR DNA Report:**

Deoxyribonucleic Acid (DNA) Analysis, employing the Polymerase Chain Reaction (PCR), was used to generate a Short Tandem Repeat (STR) profile from the following items: "list of items".[1]

Note: The following footnote will appear in all reports in which DNA testing was attempted.

[1] Loci Examined: (or Loci examined include some or all of the following) D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, and FGA.

Note: The some or all statement will be used in cases with multiple, different partial profiles. For a single partial profile the 'loci examined' statement will be used but only those loci for which data has been obtained will be listed.

Profile Match Statement [meeting the 'source attribution' criterion (estimated frequency in population of ≤ 1 in 1.6×10^{10})] for single source and identifiable major contributors of a mixture:

The DNA profile obtained from the "item description (Item #)" matches that obtained from the blood stain/sample (or reference oral swab/sample, etc.) of/from "name". Therefore, "name" is the source of the "(DNA, blood, semen, saliva etc.) " on this item[2].

Note: The following footnote will appear in any report containing the above match statement.

[2] This conclusion is based upon the following: 1) a genetic match at the gender identity locus, Amelogenin, in addition to the "number" polymorphic STR loci listed above that have an expected population frequency of less than 1 in "actual (most conservative of the population groups calculated) frequency estimate", 2) a statistical frequency exceeding the source attribution criterion of 1.6×10^{10} (for $N=1.6 \times 10^{7}$, $\alpha=0.01$; *Forensic Science Communications 2(3) July 2000)*, and 3) that "name" does not have a genetically identical twin.

Profile match Statement [not meeting the 'source attribution' criterion (estimated frequency in population of greater than 1 in 1.6×10^{10})] for single source and identifiable major contributors of a mixture:

The DNA profile obtained from the "item description (Item #)" matches that obtained from the blood/oral sample of "name". The probability of selecting an unrelated individual at random from the general population having a DNA profile that would match the DNA profile obtained from "item description (Item #)" is less than one in "actual (most conservative of the population groups calculated) frequency estimate".

artial Profile Statement [profile consistent with item(s) in match statement above]:

The DNA profile obtained from the "item description (Item #)" also matches that obtained from the blood/oral sample of "name", however less genetic information was obtained.

The partial DNA profile obtained from the "item description (Item #)" is consistent with that obtained from the blood sample of "name".

Mixture Statements:

The DNA profile from "item decription (Item#)" indicates a mixture of DNA from at least "X" persons. "Name(s)" is a potential contributor(s) to this mixture. "X%" of unrelated individuals randomly selected from the general population would be expected to be eliminated as potential contributors to this mixture.

The DNA profile from "item decription (Item#)" indicates a mixture of DNA from at least two persons. "Name(s)" is a potential contributor(s) to his mixture. The DNA profile obtained from "item decription (Item#)" is "X" times more likely to be seen if it were the result of a mixture of DNA from "name and name" than if it resulted from "name" and an unrelated individual randomly selected from the general population".

The DNA profile from "item decription (Item#)" indicates a mixture of DNA with a discernable major contributor/profile. (include match, consistent with, or exclusionary statement regarding major profile). "name" is included/excluded/cannot be excluded as a possible contributor to the minor DNA component of this mixture.

Exclusionary Statement:

The DNA profile obtained from the "item description (Item #)" does not match that obtained from the blood sample of "name". Therefore, "name" is not the source (or "a contributor" in a mixed profile situation) of the "(DNA, blood, semen, saliva etc.)" on this item.

The DNA profile obtained from the "item description (Item #)" was determined to be from an unknown male/female. "name" is not the source of the "(DNA, blood, semen, saliva etc.)" on this item.

No DNA Profile Obtained Statement:

Due to insufficient quantity or degradation, no DNA profile was obtained from "item description (Item #)".

CODIS Entry Statement:

The unknown male/female DNA profile obtained from the "item description (Item #)" was entered into the Combined DNA Index System (CODIS) to be routinely searched against the database. The submitting agency will be notified in the event of a profile match.

> *Note: This statement is included when an eligible unknown profile has been developed; however, other eligible forensic profiles will also be entered without inclusion of this statement. Eligibility of forensic profiles for entry into CODIS and upload to NDIS is according to current NDIS procedures and include both solved and unsolved cases in which the profile is associated with a crime and believed to be attributable to the putative perpetrator. Profiles matching the victim(s) and any elimination samples (e.g. consensual partner samples) may not be entered.*

11.2.7 The following statements are to be used in both **biology screening and DNA STR reports**:

Evidence Disposition Section Statements:

The following items have been retained in the laboratory [list all items/portions by description and Item# that have been retained in the DNA Packet (see BI-102)]. All remaining items have been returned to the main laboratory evidence vault for return to the submitting agency.

The following items have been forwarded for DNA analysis: [list all items/portions by description and Item# that have been retained in the DNA Packet (see BI-102)]. Results will follow in a separate report. All remaining items have been returned to the main laboratory evidence vault for return to the submitting agency.

Note: Nonsuspect cases (those with no known/identified suspect) in which biological evidence has been detected, will be forwarded for DNA testing and CODIS entry.

The DNA packet, which contains any remaining DNA extracts, has been retained in the laboratory. All remaining items have been returned to the main laboratory evidence vault for return to the submitting agency.

Evidence Description Section Examples:

A tape-sealed Sexual Assault Evidence Collection Kit (SAECK) containing biological samples, said to have been collected from "name".

A tape-sealed brown paper bag/manila evidence envelope/white cardboard box/etc. containing "description", *(include the following if collection information is known)* said to have been collected from "name" or "location".

A tape-sealed brown paper bag/manila evidence envelope/white cardboard box/etc. said to contain "label on package", *(include the following if collection information is known)* collected from "name" or "location".

A tape-sealed DNA packet, created in the laboratory on month day, year, and containing the following items:

Item #) "description"
Item #) "description"

11.2.8 It should be noted that the statements (in either the Forensic Biology Screening or DNA Reports) regarding evidence examination, testing and conclusions are not all-inclusive. There may be situations for which none of these statements is optimum.

12.0 REVIEW

Technical/administrative, document, and testimony review; as well as conflict resolution is addressed in the ISP Forensic Services Quality/Procedure Manual. See also, forms 214-BI and 306-BI in this manual.

12.1 BIOLOGY/DNA CASEWORK REVIEW

12.1.1 100% of the examinations and reports documented and/or issued from Forensic Biology/DNA will be "peer-reviewed". This review must be completed prior to issuing results (including verbal results) and/or entering eligible profiles into CODIS. Exceptions for release of results may be made on a case-by-case basis and with the Biology Supervisor's approval.

12.1.2 "Peer-review" in Forensic Biology will encompass both technical and administrative reviews.

12.1.3 The individual performing the "peer-review" will be a second scientist who is "qualified" in the area of the review (i.e., Biological Screening and/or STR Analysis).

12.1.4 It is not sufficient to have the scientist performing/reporting the analysis to be the sole person performing the administrative review.

12.1.5 The second scientist performing the review will initial each page (and date the first and last page at a minimum).

12.1.6 The second scientist will also place their initials below the signature of the scientist issuing the report.

12.1.7 Additionally, the second scientist will review the CODIS Entry Form (Form 218-BI) and verify that all eligible profiles have been identified for CODIS entry and the correct specimen categories have been assigned. The reviewer will date and initial the form.

12.1.8 Outsourced casework (when applicable) will undergo the same review as listed above, as well as for compliance with contract technical specifications.

12.2 CONVICTED OFFENDER/DATABASE SAMPLE REVIEW

12.2.1 100% of Convicted Offender sample data (including outsourced data when applicable) will be technically reviewed prior to CODIS entry and subsequent NDIS upload.

12.2.2 The individual performing the technical review will be a second scientist who is "qualified" in the area of STR Analysis.

12.2.3 The second scientist performing the review will initial each page of the data package (and date the first and last page at a minimum).

12.2.4 The scientist performing the review of outsourced data (when applicable) will document in an appropriate manner, the review of data for compliance with contract technical specifications and that the .cmf file, if present, contains the correct DNA profiles.

12.3 TESTIMONY REVIEW

Review of courtroom testimony of Forensic Biology personnel shall be accomplished at least once in each calendar year. Preferably, this review will be performed by the Biology/DNA Supervisor or another qualified analyst and documented on the Forensic Services courtroom testimony evaluation form. Alternatively, the evaluation may be completed by criminal justice personnel (i.e., the judge, prosecutor or defense counsel).

13.0 SAFETY

Laboratory safety practices are addressed in the ISP Forensic Services Health and Safety Manual. In Forensic Biology, personnel are introduced to these practices in Module 1 of the ISP Forensic Biology Training Manual. In addition, form 408B-QC (Section 8 of this manual) addresses the monitoring of the chemical eye-wash and shower.

14.0 AUDITS

Quality audits are delineated in the ISP Forensic Services Quality/Procedure Manual. Specific Biology/DNA audit requirements are delineated below.

14.1 A DNA audit, using the current FBI DNA Quality Assurance Audit Document, will be conducted on an annual basis.

14.2 The interval between annual audits will be in accordance with the current FBI Quality Assurance Standards.

14.3 Every other year, at a minimum, the DNA audit must be an external audit.

14.4 The completed audit document (Quality Assurance Audit for Forensic DNA and Convicted Offender DNA Databasing Laboratories) and appropriate accompanying documentation will be submitted to NDIS according to NDIS Operational Procedures.

15.0 OUTSOURCING

Outsourcing/Subcontracting policies and procedures are described in the ISP Forensic Services Quality/Procedure Manual.

15.1 Approved vendor laboratories must provide documentation of accreditation and compliance with the Quality Assurance Standards for Forensic DNA and/or Database Testing Laboratories prior to contract award and for the duration of the contract.

15.2 Technical specifications will be outlined in the outsourcing agreement/contract and approved (approval will be documented) by the Biology/DNA Technical Manager prior to award.

15.3 An on-site visit of the vendor laboratory will be performed, by the technical leader or a qualified DNA analyst, and documented prior to the submission of any samples to that laboratory.

15.4 An annual on-site visit will be performed and documented for any contract extending beyond one year.

15.5 When outsourcing convicted offender samples, at least one quality control sample shall be included with each batch. Additionally, at least 5% of the total outsourced samples shall be re-tested and compared for consistency and data integrity.

16.0 Practices, Methods and Forms

The following is a list of general practices/administrative procedures, analytical methods and forms utilized in Forensic Biology.

MBI≡Schemes, generally encompassing many procedures.

MBI-100	EXAMINATION OF BLOODSTAINED EVIDENCE
MBI-102	EXAMINATION OF EVIDENCE FOR SEMEN
MBI-104	EXAMINATION OF EVIDENCE FOR BODY FLUIDS
MBI-200	INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSIS

BI≡Analytical Procedures or Individual Processes

BI-100	PROCESSING LIQUID BLOOD
BI-102	DNA PACKETS
BI-104	PHENOLPHTHALEIN TEST FOR BLOOD
BI-105	O-TOLIDINE TEST FOR BLOOD
BI-106	HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST
BI-108	SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION
BI-110	BIOLOGICAL SCREENING: USE OF ALTERNATE LIGHT SOURCE
BI-114	BRENTAMINE TEST FOR ACID PHOSPHATASE
BI-116	SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION
BI-118	SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION
BI-119	SPERM DOCUMENTATION: MIDEO SYSTEM
BI-120	IDENTIFICATION OF SEMEN BY P30 DETECTION (ABAcard®)
BI-122	AMYLASE TEST: PHADEBAS
BI-124	AMYLASE TEST: STARCH IODIDE
BI-126	DETECTION OF URINE (UREASE)
BI-128	DETECTION OF URINE (CREATININE)
BI-130	DETECTION OF FECAL MATERIAL (UROBILINOGEN)
BI-200	EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS
BI-207	DNA QUANTIFICATION: REAL-TIME PCR
BI-208	STR AMPLIFICATION: PP16
BI-210	STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS
BI-301	CODIS SAMPLE RECEIPT AND DNA TRACKER ENTRY
BI-302	CODIS SAMPLE DATA ENTRY AND UPLOAD
BI-303	CODIS DATABASE HIT VERIFICATION
BI-310	CODIS SAMPLE REMOVAL

Form BI≡Various forms used for Biology Screening (1XX), DNA Analysis (2XX), CODIS (3XX) and QC (4XX) Functions.

100-BI	PHENOLPHTHALEIN REAGENT (KASTLE-MEYER)
102-BI	HYDROGEN PEROXIDE 3% (v/v)
103-BI	O-TOLIDINE REAGENT
104-BI	AMMONIUM HYDROXIDE (~3%)
108-BI	OUCHTERLONY DESTAIN
110-BI	OUCHTERLONY STAIN
114-BI	10X BRENTAMINE (SODIUM ACETATE) BUFFER
116-BI	BRENTAMINE SOLUTION A
118-BI	BRENTAMINE SOLUTION B
120-BI	SALINE (0.85% NaCℓ)
124 BI	1X PHOSPHATE BUFFERED SALINE (PBS)
126-BI	XMAS TREE STAIN SOLUTION A (KERNECHTROT SOLUTION)
128-BI	XMAS TREE STAIN SOLUTION B (PICROINDIGOCARMINE SOLUTION)
132-BI	AMYLASE DIFFUSION BUFFER (pH6.9)
134-BI	AMYLASE IODINE REAGENT
138-BI	MERCURIC CHLORIDE 10% (w/v)
140-BI	ZINC CHLORIDE 10% (w/v)
201-BI	1M TRIS-HCℓ BUFFER pH7.5
203-BI	1M TRIS-HCℓ BUFFER pH8
205-BI	ETHYLENEDIAMINE TETRAACETIC ACID (EDTA) 0.5M
207-BI	STAIN EXTRACTION BUFFER pH8
211-BI	PROTEINASE K (20 mg/mℓ)
222-BI	1M SODIUM ACETATE pH5.2
223-BI	DTT (1M)
225-BI	CHELEX REAGENT 5%
229-BI	PCR-TE (TE^{-4}) BUFFER (10mM TRIS-HCℓ, 0.1M EDTA)
231-BI	NaOH 5N
233-BI	SODIUM CHLORIDE (NaCℓ) 5M
249-BI	BOVINE SERUM ALBUMIN (BSA) 4%
101-BI	BIOLOGY SCREENING SUMMARY
200-BI	DNA EXTRACTION WORKSHEET
202-BI	DIFFERENTIAL DNA EXTRACTION WORKSHEET
206-BI	7000 LOAD SHEET
209-BI	7000 RESULTS SHEET
210-BI	STR AMPLIFICATION SET-UP
212-BI	STR BLIND CONTROL GENOTYPE CHECK
214-BI	STR TECHNICAL REVIEW CHECKLIST
216-BI	3130 LOAD SHEET
218-BI	CODIS ENTRY FORM
306-BI	STR CODIS REVIEW CHECKLIST
310-BI	CODIS SAMPLE REMOVAL CHECKLIST
400-QC	FORENSIC BIOLOGY CHEMICAL INVENTORY
401-QC	FORENSIC BIOLOGY CRITICAL EQUIPMENT INVENTORY

402-QC	FORENSIC BIOLOGY EQUIPMENT MAINTENANCE/REPAIR RECORD
403-QC	FORENSIC BIOLOGY pH CALIBRATION RECORD
404A-QC	FORENSIC BIOLOGY WEEKLY QC
404B-QC	EVIDENCE VAULT WEEKLY QC
406A-QC	FORENSIC BIOLOGY MONTHLY QC
406B-QC	FORENSIC BIOLOGY MONTHLY QC
408A-QC	FORENSIC BIOLOGY QUARTERLY QC
408B-QC	FORENSIC BIOLOGY QUARTERLY QC
410-QC	QC ABACARD® HEMATRACE® KIT
412-QC	QC ONESTEP ABACARD® P30 KIT
419-QC	QC QUANTIFILER® HUMAN DNA QUANTIFICATION KIT
420-QC	QC STR KITS
422A-QC	310 INJECTION LOG
422B-QC	3130 INJECTION LOG
426-QC	ANNUAL NIST QC RUN

MBI-100

EXAMINATION OF BLOODSTAINED EVIDENCE

1.0 BACKGROUND:

Examination of items of evidence for the presence and identification of human blood is routinely performed in Forensic Biology using visual examination, presumptive screening and confirmatory testing for identification of blood and determination of the species of origin.

Forensic Science Handbook, Chapter 7: Identification and Grouping of Bloodstains, pp.267-337, Prentice-Hall, 1982.

Sourcebook in Forensic Serology, Immunology and Biochemistry U.S. Department of Justice, NIJ, 1983 p. 73-133.

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.

2.0 SCOPE:

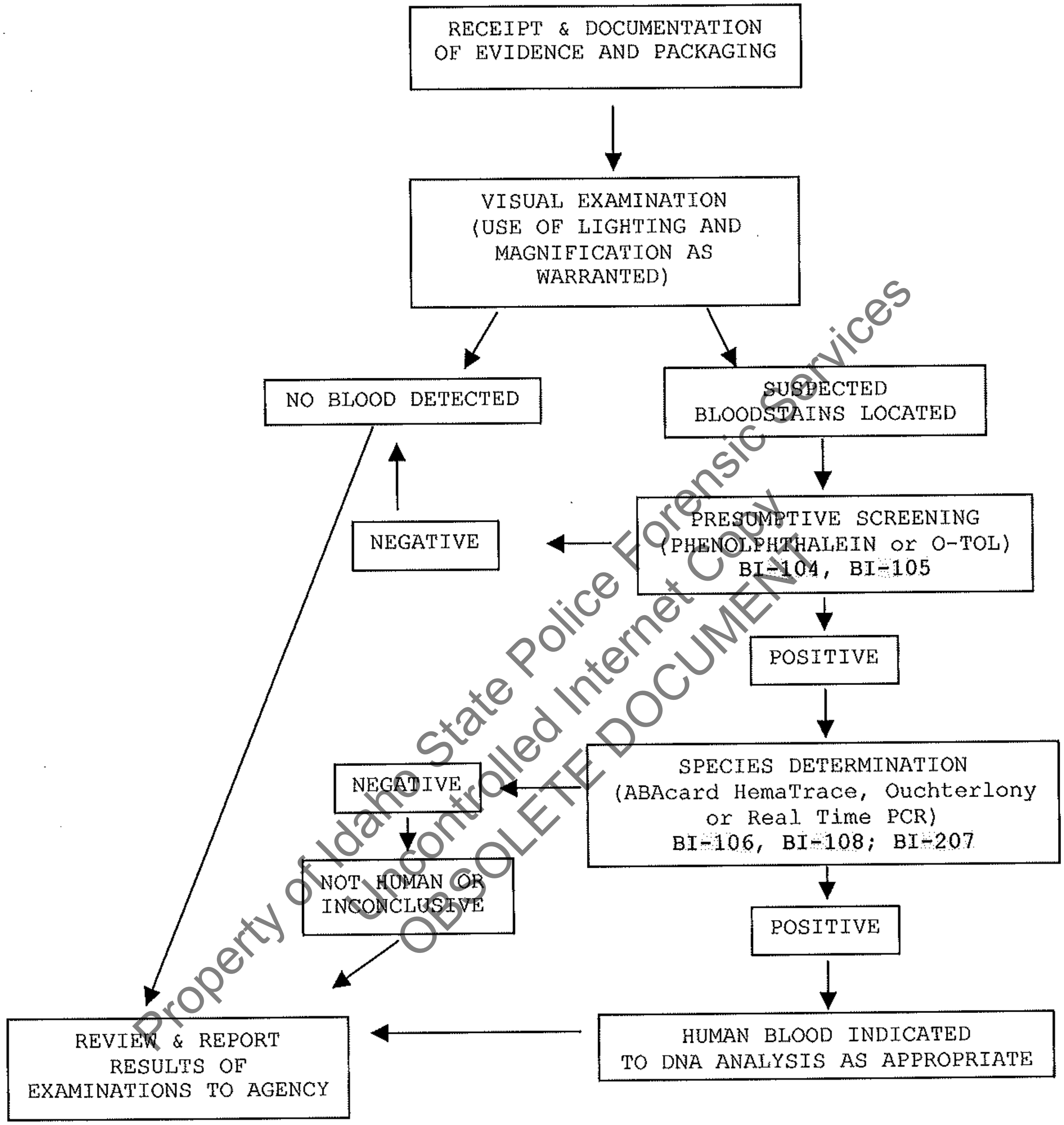
To provide uniform processing of evidentiary material for the presence of blood.

3.0 EQUIPMENT/REAGENTS:

Various lighting conditions and magnification may be used in general evidence examination to enhance the observation of blood. Reagents for blood detection and identification are listed in the appropriate processing protocols.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

5.1 In determination of species, the amount and condition of the stain should be considered in reporting a negative determination.

5.2 Discretion should be used in testing small and or poor condition samples for species determination if DNA testing is necessary.

MBI-102

EXAMINATION OF EVIDENCE FOR SEMEN

1.0 BACKGROUND:

Examination of items of evidence for the presence and identification of human semen is routinely performed in Forensic Biology using visual examination, presumptive screening and confirmatory testing for identification.

Sourcebook in Forensic Serology, Immunology and Biochemistry
U.S. Department of Justice, NIJ, 1983 p.149-181.

2.0 SCOPE:

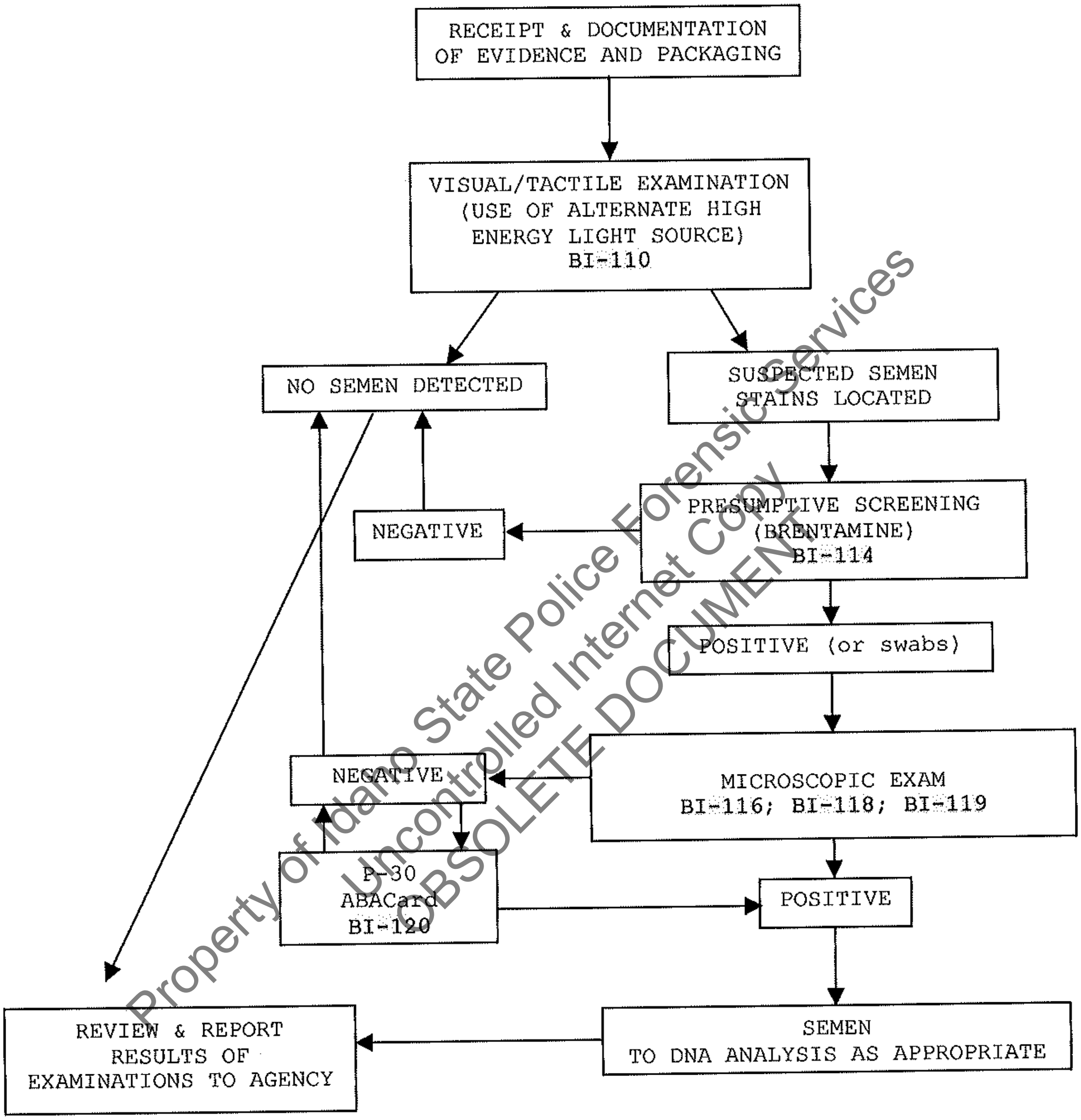
To provide uniform processing of evidentiary material for the presence of semen.

3.0 EQUIPMENT/REAGENTS:

Normal room lighting conditions and the use of an alternate light source to view fluorescence emitted from semen stains. Reagents for semen detection and identification are listed in the appropriate processing protocols.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

5.1 When examining pants/panties, a presumptive AP screening will always be performed on crotches (even in absence of visual cue).

5.2 A P-30 test need not be performed on item(s) which yielded a positive microscopic exam.

EXAMINATION OF EVIDENCE FOR BODY FLUIDS

1.0 BACKGROUND:

Examination of items of evidence for the presence of body fluids and substances other than blood or semen is sometimes requested and several methods are available to detect the presence of saliva, urine and feces.

Sourcebook in Forensic Serology, Immunology and Biochemistry U.S. Department of Justice, NIJ, 1983 pp. 197-198; 183-189; 191-195.

2.0 SCOPE:

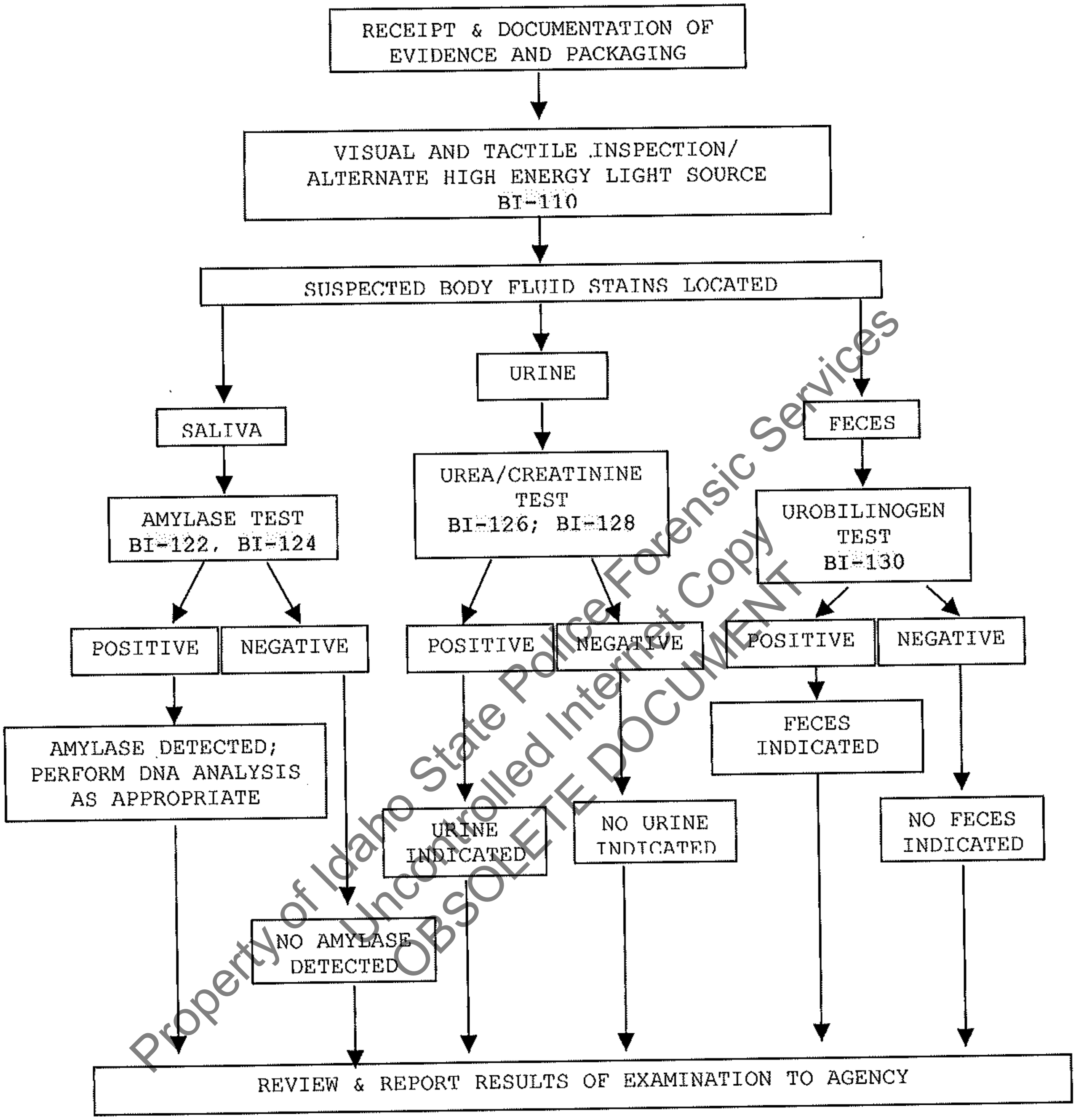
To provide uniform processing of evidentiary material for the presence of saliva, urine or feces.

3.0 EQUIPMENT/REAGENTS:

Normal room lighting conditions and the use of an alternate light source to view fluorescence and assist in the localization of possible body fluid stains. Reagents for analysis of the detected substances are listed in the appropriate processing protocols.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

5.1 Generally, feces samples and urine stains are not processed for DNA. However, exceptions may be made in instances where the sample represents the only probative evidence.

5.2 Sample size, and the significance of indicating saliva as the DNA source, should be considered before consuming sample for amylase testing.

MBI-200

INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSIS

1.0 BACKGROUND:

Once a DNA source has been detected, and identified as to 'source type' where applicable and feasible, it is often important to attribute the DNA sample to a particular individual inasmuch as possible. Current DNA technology, in the analysis of STR loci, offers individualization potential. However, the individualization of a particular sample occurs through a comparative process. This process requires a DNA profile from a 'known' sample to which the evidence sample profile can be evaluated. DNA analysis will only be performed when all necessary 'known' or 'reference' samples, for the given case, have been submitted to the laboratory.

Although the analysis of STR loci offers individualization potential, analysis may not be necessary, or performed on every case and/or sample submitted to the laboratory. DNA analysis will only be performed on cases and/or individual samples, which have the potential for resolving a probative and forensically significant question/issue regarding the given case. If the analysis of a sample resolves a given question, additional samples submitted for the resolution of the same question within the case, will likely not be analyzed. Additionally, DNA testing may establish identity, but does not establish timeframe or consent. Sexual assault cases in which consent, rather than identity, is the issue will not be analyzed for DNA.

2.0 SCOPE:

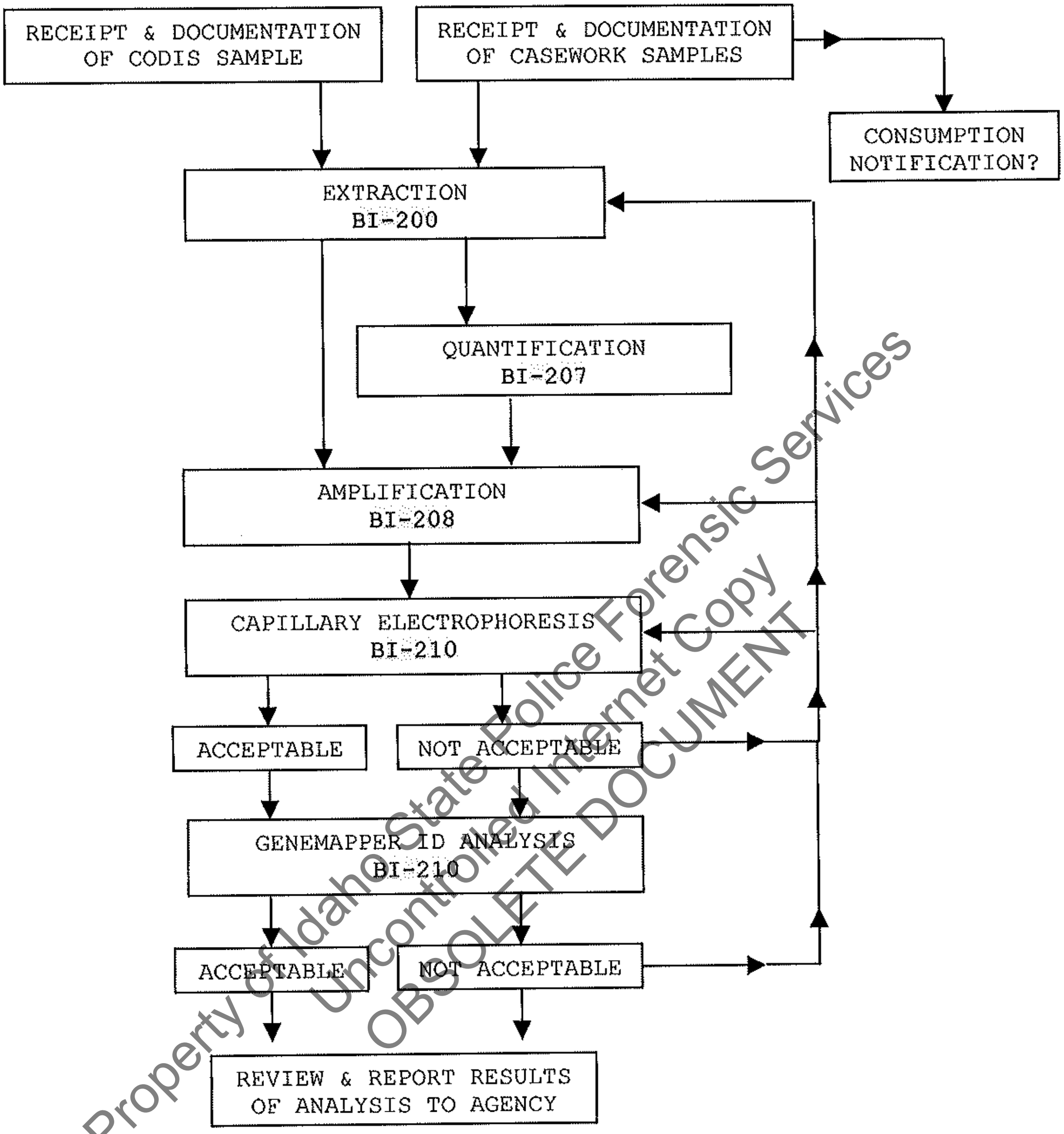
To provide uniform processing of DNA samples to achieve high quality data and consistent interpretation.

3.0 EQUIPMENT/REAGENTS:

As listed in individual analytical procedures.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

5.1 Careful scrutiny at each step will ensure insufficiencies are identified, and compensated for where feasible, at the earliest possible point (see BI-210 for specifics).

PROCESSING LIQUID BLOOD

1.0 BACKGROUND:

Most known reference standards in forensic casework are received in the form of liquid blood, generally in a lavender top (EDTA) tube. The EDTA acts as a preservative for the DNA (even up to several years post-collection); however, if left in a liquid state for prolonged periods of time (especially post-mortem samples), these samples are more susceptible to degradation, potentially resulting in the loss of DNA. These liquid samples should be stored refrigerated to aid in their preservation until which time a bloodstain can be prepared. Bloodstains stored in a dry state, even at room temperature, may be suitable for DNA testing for many years. Bloodstains are to be prepared as soon as feasible following sample receipt (generally at the time of evidence analysis). However, if evidence processing is to be delayed beyond 2 months, any post-mortem blood samples associated with the case are to be checked out and bloodstains made for preservation.

2.0 SCOPE:

To provide a method for the creation of stable DNA samples from blood.

3.0 EQUIPMENT/REAGENTS:

Blood Stain Card(s) (such as Whatman® non-FTA)
Envelopes
Disposable Transfer pipet or 1 mℓ pipet with sterile tip

4.0 PROCEDURE:

4.1 Label stain card with a minimum of Case Number, Item Number, Date and Initials. Subject name may also be placed on the card for identification purposes.

4.2 Label blood sample tube with case number, item number, date, initials, and blood level. Mix the tube thoroughly by inversion.

4.3 Remove cap from blood tube and draw ~1mℓ of blood into pipet. Carefully spot, at minimum, 1 drop (~50uℓ) blood onto each circle.

4.4 Allow bloodstain card to air-dry completely before packaging.

4.5 Place dried stain card into an envelope (~3⅛" x 5½"). Seal envelope with evidence tape on flap and label with initials and date across seal. Label front of coin envelope with Case Number and Item Number minimally.

4.6 Make Case DNA Packet (See BI-102) and place bloodstain sample inside.

5.0 COMMENTS:

5.1 Exercise caution and wear appropriate protective gear when preparing bloodstains (e.g., gloves, labcoat, protective eyewear).

5.2 Bloodstains are to be prepared either in the hood with the sash at the appropriate level, or at a workbench while wearing a disposable face shield.

5.3 Only one blood sample source should be open at a time. When processing multiple samples, close one tube before opening another and make sure stains are placed sufficiently far away from a card being processed to avoid cross-contamination.

DNA PACKETS

1.0 BACKGROUND:

It has become increasingly important to retain evidence for possible future analyses and to secure samples for nonprobative casework analyses that are necessary for the validation of any new technology. Therefore, where possible, a DNA packet is created for each case that is submitted for analysis to Forensic Biology and for which evidence exists for retention (e.g. reference sample(s) and/or positive biological screening results).

2.0 SCOPE:

To provide a method to ensure adequate sample retention for sample re-analyses and new protocol/technology development.

3.0 EQUIPMENT/REAGENTS:

Coin Envelopes (~3⅛" x 5½", and other sizes as needed)
DNA Packet Envelope (~6½" x 9¼" manila envelope)
Blue, Green, and Yellow Circular Stickers

4.0 PROCEDURE:

4.1 Cuttings/swabs containing previously identified biological evidence, as well as known reference bloodstain cards should be packaged in separate coin envelopes. Swabs packaged in separate envelopes within an outer container (sexual assault evidence collection kits, for example) do not need to be repackaged into a new coin envelope. Each envelope will be labeled with Case Number, Item Number, Date, Scientist's Initials and sealed with evidence tape.

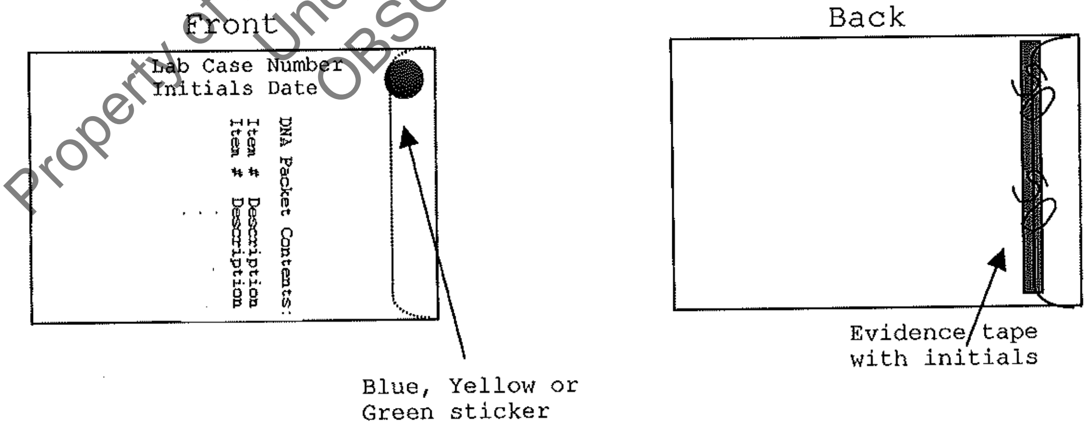
4.2 All sealed envelopes will be placed inside a larger manila envelope (DNA Packet Envelope) and labeled as below.

4.3 The DNA packet itself need not be sealed until biological screening of the case is completed and all samples are believed to have been collected.

4.4 DNA Packets for crimes without a statute of limitations (i.e., Homicides, and Sexual Assaults where DNA evidence exists, including references for criminal paternity testing, and nonsuspect/database cases) will be identified by placement of a blue circular sticker on the outside of the DNA Packet (see below). Likewise, cases that have negative biological screens (so that the DNA Packet will consist solely of the reference bloodstains, except criminal paternity cases) will be identified by the presence of a yellow circular sticker. Green stickers will be placed on the DNA Packets of all other cases.

4.5 Once sealed, the DNA Packet will be taken to a FES and entered as an additional item of evidence to allow for tracking in the ETS. The storage location will have a barcode.

4.6 DNA Packets will be stored at ≤ -20°C as space allows, and then, if necessary, either returned to the submitting agency, or placed in room temperature storage after any requested DNA analyses have been performed. However, prior to return to a submitting agency, the Biology/DNA Supervisor should be notified to ensure maintenance on site is no longer necessary.



4.7 Following DNA testing, any leftover DNA extracts will be put into a plastic ziplock bag or coin envelope and placed in the DNA Packet. Individual tubes may also be sealed with parafilm or other sealant to prevent leakage and/or evaporation if desired.

5.0 COMMENTS:

5.1 The DNA Packet is NOT meant to contain "items of evidence" but rather biological samples that have been removed from items of evidence. Not every item or every stain on every item should be included in a DNA Packet. The person performing the biological screening should use discretion and prioritize sample collection contacting a DNA Analyst or the Biology Program Manager if necessary.

5.2 Given the small sample necessary for DNA testing, discretion should be used in determining the size of the stain cutting. Rarely, if ever, should a cutting exceed the dimensions of the coin envelope.

5.3 On **RARE** occasions when it is deemed necessary to have more stains collected in a given case than will fit into a single DNA Packet Envelope, multiple packets will be made. The first packet's barcode will consist of the case number followed by DNA. Subsequent packets will receive barcodes consisting of the case number followed by DNA2, DNA3, etc.

PHENOLPHTHALEIN TEST FOR BLOOD

1.0 BACKGROUND:

Most screening tests for blood depend on the catalytic action of the heme group. To minimize false positives, the test is frequently performed as a multi-step test. A good overview is found in the first reference.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* (1983) U.S. Dept. of Justice, Washington, D.C., p. 101-105.

Higaki, R.S. and Philp, W.M.S. *A Study of the Sensitivity, Stability and Specificity of Phenolphthalein as an Indicator Test for Blood,* (1976) Canadian Journal of Forensic Science, Vol 9, No.3, p.97-102.

2.0 SCOPE:

To provide a method for the localization and presumptive identification of bloodstains.

3.0 EQUIPMENT/REAGENTS:

Phenolphthalein Working Solution
3% Hydrogen Peroxide
Sterile/Nanopure H_2O
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

4.1 Positive (known bloodstain) and negative (sterile/nanopure H_2O) control samples are processed, prior to testing any forensic samples (on the day of testing), to ensure the working solution reagents are functioning properly.

4.2 Cotton swabs or a folded piece of filter paper are used to collect the suspected blood onto the tip. A swab may be moistened with sterile/nanopure H_2O if necessary.

4.3 To the swab or filter paper with the suspected blood, add 1-2 drops of phenolphthalein working solution. Wait 10-15 seconds to detect potential false positives.

4.4 Add 1-2 drops of 3% H_2O_2 and note appearance or absence of bright pink color. Color reaction should occur rapidly (≤ 1 minute).

4.5 Document result in case notes. Record positive (+), as indicated by the development of the above color change, or negative (-) as indicated by the absence of the color change. Analyst may use other descriptive word(s) as well (e.g., strong, weak, slow, etc.).

5.0 COMMENTS:

5.1 Direct testing of a small cutting/sample may also be performed.

5.2 Color changes occurring prior to the addition of 3% H_2O_2 are generally considered inconclusive.

5.3 Color changes occurring after 1 min. are generally considered negative.

O-TOLIDINE TEST FOR BLOOD

1.0 BACKGROUND:

Most screening tests for blood depend on the catalytic action of the heme group. To minimize false positives, the test is frequently performed as a multi-step test. A good overview is found in the first reference.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p. 101-105.

Burdett, PE (October 1976) "Presumptive Tests for Blood - A Comparative Survey", *CRE Report, No. 201*.

Culliford, BJ and Nicholl, LC (1964) "The Benzidine Test: A Critical Review", *Journal of Forensic Sciences*, 9:175-191.

2.0 SCOPE:

To provide a method for the localization and presumptive identification of bloodstains.

3.0 EQUIPMENT/REAGENTS:

0.3% Ortho-Tolidine Stock
3% Hydrogen Peroxide
Sterile/Nanopure H_2O
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

4.1 Positive (known bloodstain) and negative (sterile/nanopure H_2O) control samples are processed, prior to testing any forensic samples (on the day of testing), to ensure the working stock reagents are functioning properly.

4.2 Cotton swabs or a folded piece of filter paper are used to collect the suspected blood onto the tip. A swab may be moistened with sterile/nanopure H_2O if necessary.

4.3 To the swab or filter paper with the suspected blood, add 1-2 drops of o-tolidine working solution. Wait 10-15 seconds to detect potential false positives.

4.4 Add 1-2 drops of 3% H_2O_2 and note appearance or absence of blue-green color. Color reaction should occur rapidly ($\leq$ 1 minute).

4.5 Document result in case notes. Record positive (+) as indicated by the development of the above color change, or negative (-) as indicated by the absence of the color change. Analyst may use other descriptive word(s) as well (e.g., strong, weak, slow, etc.).

5.0 COMMENTS:

5.1 Direct testing of a small cutting/sample may also be performed.

5.2 Color changes occurring prior to the addition of 3% H_2O_2 are generally considered inconclusive.

5.3 Color changes occurring after 1 min. are generally considered negative.

5.4 O-tolidine is designated as a potential carcinogen and should be used with caution.

HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST

1.0 BACKGROUND:

Items of evidence with unknown sources of blood are often submitted in forensic casework and it is useful to be able to determine whether the blood is of human origin. The basis of the ABACard® Hematrace® test is the immunological detection of human hemoglobin.

2.0 SCOPE:

To provide a uniform and reliable method for confirming the presence of blood on evidentiary material.

3.0 EQUIPMENT/REAGENTS:

OneStep ABACard® Hematrace® Test Kit

4.0 PROCEDURE:

4.1 Label extraction tubes for identification.

4.2 Using the buffer provided, allow samples (generally ~2mm x 2mm stain cutting) to extract at room temperature for 5-30 minutes (longer, if necessary for aged stains).

4.3 Label an ABACard® Hematrace® test device for each sample, including controls.

4.4 Apply ~150µℓ (4 drops with provided dropper) of a sample extract to the 'S' well of its corresponding test device and incubate at room temperature for ≤ 10 minutes.

4.5 A positive result is indicated by the appearance (within 10 minutes) of a pink line in both the control 'C' and test 'T' areas. A negative result is indicated by the absence of a pink line (after 10 minutes) in the 'T' area of a test device. Results are inconclusive anytime a pink line fails to develop in the 'C' area.

5.0 COMMENTS:

5.1 Samples must be at room temperature for the test. If extracts have been stored in refrigerator/freezer, allow them to reach room temperature before proceeding.

5.2 Both positive (known human bloodstain) and negative (extraction buffer alone) controls are used.

5.3 Since the reaction time is dependent on hemoglobin concentration, as well as other sample-specific factors, it is necessary to wait the full 10-minute incubation before reporting a negative result. However, a positive reaction may occur in much less time.

5.4 As with any antigen-antibody reaction, false negatives (as the result of a "high dose hook effect") may be produced with concentrated samples. When negative results are obtained with very 'heavy' stains, the sample should be further diluted and the test repeated.

5.5 Other reagents may be used for extraction. For example, 3-5% Ammonia Hydroxide (aged stains), saline, 1XPBS or PCR-TE. The volume used for extraction may be reduced for sample conservation or dilute stains (e.g., 150µℓ).

5.6 Although most nonhuman species tested do not produce a positive result with the ABACard® Hematrace® test, some crossreactivity has been reported (i.e., other primates, weasel, ferret, skunk). Therefore, when reporting results, the statement 'indicated the presence of human blood' should be used, rather than 'detected' or 'identified'. In instances where species crossreactivity may be plausible, a statement indicating that 'members of the mustelidae family cannot be excluded' may also be used in the report.

SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION

1.0 BACKGROUND:

Methods commonly used to identify the species of origin of a biological sample are immunological in nature. The Ouchterlony Double Diffusion technique was first described in 1949 and involves the diffusion of antibody (Ab) and antigen (Ag) in an agarose gel. The formation and detection of a precipitin line (as the result of Ab-Ag complex formation) is used to determine the species of origin of a particular sample.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., pp. 101-105.

Saferstein, R. *Forensic Science Handbook* (1982) pp.284-297.

2.0 SCOPE:

In forensic biology, it is usually the determination of whether a bloodstain is of human origin that is of concern. That determination will generally be made using the ABACard® Hematrace® test. However, there may be instances where it is important to determine what nonhuman species was the source of a given sample or whether a nonblood sample is of human origin. In those situations this method may be used and is limited only by the availability of specific antisera and positive control materials (this method may also be used in place of the ABACard® Hematrace® test for the identification of human blood).

3.0 EQUIPMENT/REAGENTS:

3% Ammonium Hydroxide (for aged stains)
Antisera
Agarose, E25 or Sigma Type I
Glass Microscope Slide(s) (5 x 7.5 cm)
GelBond® (cut to 5 x 7.5 cm)
Agarose Punch or equivalent (e.g., pipet and vacuum)

1M NaCℓ
2% Coomassie Blue Stain and Destain solutions
filter paper

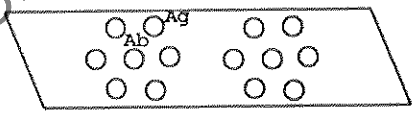
4.0 PROCEDURE:

4.1 Extract a small sample (e.g., $2mm^2$ bloodstain) in ~50 μℓ dH_20 (or 3% Ammonium Hydroxide for aged bloodstains). Bloodstain extracts should be somewhat dilute and straw-colored in appearance. Extraction time and dH_2O volume will vary depending on stain concentration in order to achieve the desired straw color supernatant.

4.2 In order for the agarose to sufficiently adhere to a microscope slide, GelBond® must be adhered to the slide and the agarose gel formed on top of it. Cut GelBond® to the approximate size of your microscope slide and adhere hydrophobic side to slide with a few drops of dH_20.

4.3 Prepare a 1% agarose gel by boiling 0.8 g agarase in 8 ml dH_2O. Carefully pour agarose gel onto hydrophilic side of the GelBond®. Allow solidification of gel.

4.4 Using a pre-made Ouchterlony punch or pipet/pipet tip with vacuum, create a pattern of Ag wells around a central Ab well as depicted below (~3mm between Ab and Ag wells) in the solidified agarose.



4.5 Pipet appropriate antisera into central well(s) and sample extract(s) (include a positive control of interest and an extraction reagent blank; substrate control where appropriate) into surrounding well(s).

4.6 Allow immunodiffusion to take place overnight, at room temperature, in a moisture chamber (enclosed vesicle with dH_20-moistened paper towel, filter paper, or sponge).

4.7 Precipitin bands at this stage are best viewed with strong backlighting against a dark background. The immunodiffusion gel should be soaked, dried and stained for enhanced visualization.

4.8 Staining

4.8.1 Soak immunodiffusion gel in 1M NaCℓ for ≥ 6 hours (may be left overnight) to remove uncomplexed proteins.

4.8.2 Rinse the gel in dH_20 for ~5 minutes; dampen two pieces of filter paper with dH_20 and place on top of gel, followed by a stack of paper towels to serve as a wick. Place a weight on top of the paper towels to 'press' the gel for ≥ 30 minutes. Remove the weight, paper towels, and filter paper and dry the gel in an oven at 56°C-65°C for ≥ 20 minutes.

4.8.3 Immerse gel in Stain Solution for 10-15 minutes.

4.8.4 Destain until background is clear and blue precipitate bands can easily be seen.

4.8.5 Rinse with dH_2O and allow to dry.

5.0 COMMENTS:

5.1 A clear, distinct precipitin band between the antisera well and sample well is a positive test result. Extraction blanks should be negative (i.e. no precipitin band present).

5.2 "Spurs" may be seen on precipitin bands produced from closely related species.

5.3 Note: the gel/GelBond will separate from the glass slide at some point, however, the gel should remain in contact with the GelBond.

BI-110

BIOLOGICAL SCREENING: USE OF ALTERNATE LIGHT SOURCE (ALS)

1.0 BACKGROUND:

There are numerous forensic applications for the use of alternate lighting. In forensic biology, it is generally used to aid in the visualization of physiological fluids and trace evidence such as fibers.

2.0 SCOPE:

To provide a method for enhancing visualization/localization of physiological fluids and trace evidence (as necessary for preservation) on evidentiary items.

3.0 EQUIPMENT/REAGENTS:

Alternate Light Source
Filtered Safety Goggles

4.0 PROCEDURE:

4.1 Selection of the wavelength of light for viewing will depend on the alternate light source used and its available outputs. A broadband source covering ≤530nm wavelengths is sufficient for biological examination but will not eliminate potential background fluorescence as well as the use of a discrete wavelength band. Optimum visualization of physiological fluids and fibers is achieved at ~450nm and ~485nm, respectively. The following table illustrates the appropriate safety goggles to be used with various source outputs.

Wavelengths	**Safety Goggles**
< 400 (UV)	Yellow/UV safe
< 530 broadband	Orange
400-450 discrete	Yellow
450-540 discrete	Orange
540-700 discrete	Red
700-1100 discrete >700 broadband	Red or IR safe

4.2 Follow manufacturer's operating instructions for specific details on equipment operation.

4.3 Examine evidence under optimum discrete wavelengths where possible and under appropriate broadband output when discrete wavelengths are not available.

4.4 Mark any fluorescent areas/potential biological stains, as appropriate and necessary, for ease of location under normal room lighting conditions.

4.5 Proceed to appropriate screening procedure(s) for any possible biological stains located (see BI-114; BI-122; BI-124; BI-126; BI-128)

5.0 **COMMENTS**:

5.1 Failure to use safety goggles, or use of incorrect goggles could result in permanent eye damage. Avoid looking into the wand or shining on other individuals. Read any manufacturer's safety guidelines provided with the equipment.

5.2 Ultraviolet light may cause burns, so caution should be exercised to avoid direct and/or prolonged exposure to unprotected skin. Read any manufacturer's safety guidelines provided with the equipment.

5.3 The alternate light source wand can generate heat and potentially cause burns to skin and other materials. Read any manufacturer's safety guidelines provided with the equipment.

BI-114

BRENTAMINE TEST FOR ACID PHOSPHATASE

1.0 BACKGROUND:

Acid phosphatase is an enzyme found in elevated amounts in human semen, independent of the presence of spermatozoa. Various tests have been used for its detection. Though none of these tests are prostate-specific, at the limits of their detection, they are a good indicator of the presence of semen.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* (1983) U.S. Dept. of Justice, Washington, D.C., p 155-166.

Biology Methods Manual, Metropolitan Police Forensic Science Laboratory, p.3-16 through 3-19.

2.0 SCOPE:

To provide a method to presumptively identify the presence of semen and an aid to be used in locating semen stains.

3.0 EQUIPMENT/REAGENTS:

Brentamine Solution A
Brentamine Solution B
Sterile/Nanopure H_2O
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

4.1 Prepare Brentamine Working Stock: Mix 1 part solution A and one part solution B with 8 parts of water. This solution should be prepared fresh each day it is used.

4.2 Positive (known semen stain) and negative (moistened swab or filter paper) control samples are processed, prior to testing any forensic samples (on the day of testing), to ensure the working stock reagents are functioning properly.

4.3 Lightly rub a suspected semen stain with a pre-moistened cotton swab, or press a moistened piece of filter paper against the stain.

4.4 Add Brentamine Working Stock to the swab or filter paper and observe for the appearance or absence of a pink to purple color change.

4.5 To avoid false positives, the results should be recorded as positive(+), as indicated by the development of the above color change, or negative(-), as indicated by the absence of the color change, within 1 minute of the addition of the Brentamine Reagent. Additional comments (e.g., strong, weak, slow, etc.) may also be helpful to record.

5.0 COMMENTS:

5.1 Positive reactions, though generally weak, may be obtained on anal/rectal and some vaginal swabs in absence of any semen.

5.2 The test may also be performed using 10-20µℓ of a sample extract or directly onto a small cutting.

5.3 This test may also be used for mapping large, possible semen stains via a moistened paper transfer method. A sheet(s) of moistened filter paper is pressed against the item of evidence. Marks are made on the paper to indicate the edges of the evidence for orientation of any subsequent color reaction. The paper is sprayed with Brentamine Reagent and analyzed as above.

5.4 Fast Blue B is a possible carcinogen and should be handled cautiously.

BI-116

SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION

1.0 BACKGROUND:

The identification of semen is a multi-step process for which it is necessary to generate extracts of putative semen stains for use in the identification tests.

2.0 SCOPE:

To provide a method of generating suitable extracts from evidentiary material for the performance of both presumptive (as needed) and confirmatory testing for the presence of semen, as well as other forensic analyses.

3.0 EQUIPMENT/REAGENTS:

Small (e.g., 12x75mm) tubes **or** 1.5/0.5mℓ microfuge tubes
Centrifuge

4.0 PROCEDURE:

4.1 Label tubes with identifying information.

4.2 Take a sample (~3-5 mm^2 portion of stain or ~1/8 each of one or two cotton swabs), transfer to the appropriately labeled tube and extract in a minimal volume (50µℓ - 100µℓ) of dH_2O at RT for ≥ 20 minutes.

4.3 At this point, agitation, vortexing, brief sonication and/or piggyback centrifugation may be used to assist in removing sperm/cellular material from the substrate.

4.4 Mix/resuspend the sample for use in microscopic examination (BI-118; BI-119) and/or p30 detection (BI-120). Alternatively, the supernatant may be removed, without disturbing the pellet, for additional testing [e.g. AP screening (BI-114), p30, etc.] prior to resuspension.

5.0 COMMENTS:

5.1 Other reagents may be substituted for dH_2O (e.g., 1XPBS, PCR-TE, saline) in 4.2.

5.2 The sample sizes and extraction volumes are those typically used and are recommendations. The scientist has the discretion to increase or decrease the sample size and corresponding extraction volume as case circumstances dictate.

5.3 While the primary use of this liquid extract is for semen identification testing, these extracts may be used for other screening tests as well (e.g., saliva, urine, feces).

5.4 The sample may optionally be extracted in dH_2O directly on the microscope slide at the analyst's discretion. However, the quantity of sperm observed may be diminished and no sample will remain for further testing (e.g. p30) when using this method.

SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION

1.0 **BACKGROUND**:

The visual identification of spermatozoa is a means of positively identifying human semen. Human sperm have a distinctive size and morphology and, with differential staining, such as the "Xmas Tree" method, can be readily identified.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., pp. 150-152.

2.0 **SCOPE**:

To provide a confirmatory test for the identification of semen in cases where spermatozoa are present.

3.0 **EQUIPMENT/REAGENTS**:

XMas Tree Stain Solution A
XMas Tree Stain Solution B
≥95% Ethanol
Glass Microscope Slide(s)
Cover Slip(s)
Mounting Medium
Microscope (Magnification ~200X-400X)
Mideo System

4.0 **PROCEDURE**:

4.1 The sample extract is mixed well and ~20-50μℓ deposited on a microscope slide and allowed to dry (this process may be expedited by use of a slide warmer or oven at ~37°C).

4.2 Heat-fix the sample extract to the slide by slowly passing over a flame (alcohol lamp or Bunsen burner).

4.3 Cover the heat-fixed sample extract with Xmas Tree Stain Solution A and allow staining for ≥ 15 minutes at RT.

4.4 Remove the stain with a gentle stream of dH_2O and cover the stained area briefly (~15-20 seconds) with Xmas Tree Stain Solution B. Remove this stain with a stream of EtOH (95% or Absolute).

4.5 Allow the slide to dry and apply mounting medium or dH_2O and a cover-slip prior to microscopic examination.

4.6 Scan the slide on ≥200X magnification. Sperm heads will retain the red stain, while the tails, if present, will appear green. Use 400X magnification if necessary to verify sperm morphology. The Mideo System may be used as an alternate scanning method, or as a means of documentation only (see BI-119).

4.7 Documentation in notes should include the following:

4.7.1 A description of the condition of the sperm seen (e.g. heads only, mostly heads, some intact, etc.).

4.7.2 An estimate of the number of sperm seen per field (e.g., 12/slide; 0-1/200X; 3-5/200X; 5-10/200X; >10/200X; or 1+ - 4+ etc.). A representative photograph depicting the overall rating of the slide shall be taken and included in the note packet (see BI-119).

4.7.3 The presence of any epithelial cells (e-cell) and their number (e.g., rare, occasional, few, moderate, many, or 1+ - 4+). The scientist may also note e-cell descriptions [e.g. nucleated (NEC or nuc.) or anucleated (ANEC or Anuc.)] and whether or not there are large squamous epithelial cells present.

4.7.4 If the situation arises in which there are only one to three sperm heads, a single intact sperm, or a few sperm heads of questionable morphology, a second qualified scientist must verify the identification. A photograph of the single sperm shall be taken and included in the note packet (see BI-119).

4.7.5 For ease of re-location, the position of sperm in cases where 3 or less have been identified should be documented in the case notes.

4.7.6 It is also good, if possible, to note the presence of significant amounts of bacteria, yeast or white blood cells.

5.0 COMMENTS:

5.1 Stains purchased commercially have expiration dates, while those prepared 'in-house' are generally stable for ≈ 6 months at RT. After this period, stains should be discarded or checked with a positive (known sperm) slide before use.

SPERM DOCUMENTATION: MIDEO SYSTEM

1.0 BACKGROUND:

The presence of semen may be confirmed by microscopic identification of spermatozoa. The Mideo System allows microscopic images to be visualized on a computer screen, captured, stored and printed. The EZDoc software allows for tracking of images and any modifications. A report with a representative slide view or views of individual spermatozoa will be included in the case file when sperm are identified.

EZDoc Plus Administrator/User Manual.

2.0 SCOPE:

To provide a means of confirming and documenting the presence of semen in cases where spermatozoa are present; as well as other photographic documentation and/or storage.

3.0 EQUIPMENT/REAGENTS:

Imaging Computer with Flat Screen LCD
EZDoc Plus Case Image Management Software
BX-45 Ergonomic Microscope (Magnification ~200X-400X)
Digital Microscope Camera
Touch Screen and Controller
Printer

4.0 PROCEDURE:

4.1 SPERM SEARCH

Note: Refer to BI-118 for slide preparation.

4.1.1 Turn on the computer, monitor, microscope and touch screen controller.

4.1.2 Double click the EZDoc Plus icon on the desktop. Enter a User Name and Password, select a role (typically scientist), select a previously used Case ID or choose "New" to enter a new case, enter the Evidence ID, click "OK"; the window will display a live video image.

4.1.3 Choose "GO TO LOAD" on the controller screen and place microscope slide in the slide holder with frosted portion on the right. Choose "GO TO HOME" and the slide will move under the oculars.

4.1.4 Search the slide at 200X magnification using the joystick or select a pattern from the controller screen (use 400X magnification if necessary to verify sperm morphology). To start a pattern, select the 'Pattern' tab, 'Run' tab, then highlight a pattern and press "RUN".

4.1.5 Focus using the knob on the right side of the joystick controller or the "FOCUS UP"/"FOCUS DOWN" buttons on the controller screen.

4.1.6 Navigate the pattern scan using the "PAUSE", "CONTINUE", "BACK" and "NEXT" buttons on the controller screen.

4.1.7 To save a point(s) during a pattern scan, press "SAVE" while the image is in view.

Note: This step saves points on the controller screen for the current scan pattern. It does not save the points or images in the EZDoc Plus software. See Image Capture below to save images within a case file.

4.1.7.1 To review saved images, go to the Stored Points tab; highlight the point(s), choose "OK", highlight a point and press "GO TO".

4.1.7.2 To delete saved points, go to the Delete tab; choose "POINTS", highlight the point and press "DELETE". **Do not choose "DELETE ALL".**

4.2 IMAGE CAPTURE

4.2.1 Use the "Live Input" white screen icon on the tool bar to toggle between a still image and live input.

4.2.2 To save the image, click "File-Save Image" or the "Save Image" disk icon on the toolbar. When the dialog box appears, specify the image file name and description, click "OK".

4.2.3 Click "Check Out" to add overlays or make changes to the image.

4.2.4 Click "Forms-Forms" and "Tools-Overlays" or the Overlays toolbar icon to add text, graphics, scales, timestamp, etc.

4.2.5 Click "Check In" when finished modifying the image and note changes when prompted.

4.2.6 If only one to three sperm are present, the image(s) must be peer reviewed by another Scientist prior to printing a report. Highlight the image(s) to be reviewed in the Visual Directory and click "Submit For Review"; an icon with a blue mark will appear next to the image. Notify the reviewing Scientist of the images to be reviewed and their location.

4.3 REPORT

4.3.1 Highlight image(s) from the Visual Directory to be printed (under "DB Images" in the file menu), click "Report", select report type and click "Print".

4.3.1.1 Select "Single Image Report" for an image with descriptions and notes.

4.3.1.2 Select "Four Up Report" to display up to four smaller images per page.

4.4 END SESSION

4.4.1 Exit out of the EZDoc Plus Software.

4.4.2 Choose "GO TO LOAD" on the controller screen and remove the microscope slide.

4.4.3 Backup the database by double clicking on the Maxtor OneTouch icon, click 'Sync', followed by 'Sync Now'. A message will appear when complete. Choose 'OK'.

4.4.3 Turn off the computer and monitor using "Start-Shut Down" in the bottom left corner and select "Turn Off Computer".

4.4.4 Switch off power to the microscope and touch screen controller.

4.4.5 Return the mouse to the cradle.

4.5 PEER REVIEW

4.5.1 Logon to EZDoc Plus as before and choose the case ID to be reviewed.

4.5.2 Select "DB Images" from the file menu. Select the case, evidence and user from the image location directory. Double click on the thumbnail image(s) with a blue mark, perform the review, add notes, etc.

4.5.2.1 To approve an image, select "Accept"; the Peer Reviewer's ID will be added and a red mark will appear on the icon.

4.5.2.2 For a questionable or rejected image, notify the creating Scientist of the objections and/or comments. The creating Scientist may then hit "Reject/Unsubmit" to return the item to its original state.

5.0 COMMENTS:

5.1 Original images and a record of all changes are maintained in the History and reports are available.

5.2 Do not press "DELETE ALL" on the controller screen or all of the patterns will be deleted.

5.3 While viewing a live image, the X, Y and Z coordinates are displayed on the controller screen Digital Read Out tab; to document the point coordinates add a text overlay or notes to the image.

5.4 To improve the image, a filter may be engaged by placement in the microscope filter slider. The brightness, contrast, and zoom enhancement features are also available via the Enhance menu.

5.5 Casework and crime scene photographs may also be imported, saved and stored. Images can be copied to a local directory or imported directly from a CD, disk, camera, or memory card. Go to "File-Local Images", click on the desired image(s) and select "Batch Move to DB" (files may be moved individually, if desired). A new window will appear, which allows you to rename each image or capture the current names. A report may be printed for the case file as above. Large numbers of photographs (typically associated with crime scenes) can be printed to a proof sheet; from the Visual Directory, click "Select All" and "Print Proof Sheet". This process may be lengthy for numerous photos.

5.6 The database should be backed-up (as above) following each user session.

BI-120

IDENTIFICATION OF SEMEN BY P-30 DETECTION (ABAcard®)

1.0 BACKGROUND:

P-30 is a seminal-fluid-specific protein. Its presence in semen is independent of the presence of spermatozoa. Immunological detection of p30 is commonly used as a confirmatory test for the presence of semen.

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

Spear, T. F. and Khoskebari, N. The Evaluation of the ABAcard® p30 Test for the Identification of Semen. (2000) *Crime Scene*, 26(1): 9-12.

2.0 SCOPE:

This procedure is to be used as a confirmatory test for the presence of human semen in instances where a positive AP result was obtained but no spermatozoa were seen upon microscopic examination of the sample extract.

3.0 EQUIPMENT/REAGENTS:

OneStep ABAcard® p30 Test Kit

4.0 PROCEDURE:

4.1 Label an ABAcard® p30 test device for each sample, including controls.

4.2 Add 10µℓ of each sample(see BI-116), to include both positive (known semen stain extract or Seri™ semen standard [10ng; 10µℓ of a 1:100 dilution]) and negative (saline) controls, to ~190µℓ (4 drops) of saline and mix thoroughly.

4.3 Transfer each extract (~200µℓ) to the 'S' well of the appropriately labeled test device and incubate at RT for 10 minutes.

4.4 A positive result is indicated by the appearance (within 10 minutes) of a pink line in both the control 'C' and test 'T' areas. A negative result is indicated by the absence of a pink line (after 10 minutes) in the 'T' area of a test device. Results are inconclusive anytime a pink line fails to develop in the 'C' area.

5.0 COMMENTS:

5.1 Samples must be at room temperature for the test.

5.2 Other reagents may be substituted for saline (e.g., 1XPBS, PCR-TE, dH_2O) in 4.2.

5.3 Since the reaction time is dependent on p30 concentration, as well as other sample-specific factors, it is necessary to wait the full 10-minute incubation before reporting a negative result. However, a positive reaction may occur in much less time.

5.4 As with any antigen/antibody interaction, excess antigen may lead to a 'high dose hook effect' resulting in false negatives when the p30 concentration is very high. This effect should be considered when examination and presumptive tests have indicated the likelihood of the presence of semen. In those instances, the sample should be diluted and the test repeated.

AMYLASE TEST (PHADEBAS)

1.0 BACKGROUND:

Amylase is an enzyme that is present in high concentrations in saliva relative to other body fluids and its detection is indicative of the presence of this body fluid. This method for the detection of amylase consists of a tablet of water-insoluble starch, cross-linked to Cibacron Blue dye, that is hydrolyzed to water-soluble blue fragments in the presence of alpha-amylase and detected by blue color development of the solution.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p 184-187.

Auvdel, Michael J. Amylase Levels in Semen and Saliva Stains, (1986) *Journal of Forensic Sciences*, 31 (2) 426-431.

Keating, S.M. and Higgs, D.F. The detection of amylase on swabs from sexual assault cases, (1994) *Journal of the Forensic Science Society*, 34 : 89-93.

G.M. Willott, "An Improved Test for the Detection of Salivary Amylase in Stains," *Journal of the Forensic Science Society*, 14, pp. 341-344 (1974).

Phadebas Amylase Test directions for use, Pharmacia AB, Uppsala, Sweden, 1994 and Magle AB, Lund, Sweden, 2007.

2.0 SCOPE:

To provide a presumptive screening test for the presence of saliva on evidentiary items.

3.0 EQUIPMENT/REAGENTS:

Phadebas Tablets
0.5N NaOH
Sterile/Nanopure H_2O
12x75mm tubes

Corks for tubes or parafilm™ or equivalent

4.0 PROCEDURE:

4.1 Stain samples (~2-5mm^2; ⅛-¼ swab; 20μℓ extract) and controls [20μℓ dH_2O is used for negative control; 20μℓ of 1:100 and 1:500 dilutions of fresh saliva and either neat saliva, or a saliva stain (≤2mm^2 cutting) as positive controls] are placed into appropriately labeled tubes.

4.2 Add 1mℓ dH_2O and 1/4 Phadebas tablet to each tube, cover tube, mix well (e.g. vortex) and incubate at 37°C for 30 minutes.

4.3 At RT, remove cork, add 250μℓ 0.5N NaOH to each tube, cover tube, mix well by inversion and spin for 5 minutes at low speed (<5,000 rpm).

4.4 Examine tubes and record the color of the supernatant. The intensity of the blue color, if present, may be graded as light, medium, dark, or 1^+-4^+. For reporting, see **5.1**.

5.0 COMMENTS:

5.1 If the blue color of a sample is as dark or darker than that of the 1:500 control, it is an indication of an elevated level of amylase and is reported as such. If the blue color of a sample is lighter than the 1:500 control, there is an indication that amylase is present; however, there is no demonstration of an elevated level. A sample that demonstrates absence of blue color consistent with the negative control is reported as 'did not indicate the presence of amylase'. Note: negative samples (like the control) may have a very slight blue tint and not appear perfectly clear.

5.2 A negative result is not necessarily the total absence of saliva, and therefore, DNA testing should not be abandoned because of the absence of detectable amylase activity.

5.3 This test is not human specific, there may be reactive amylases in plants and non-human animals.

BI-124

AMYLASE TEST (STARCH IODIDE)

1.0 BACKGROUND:

Amylase is an enzyme that is present in high concentrations in saliva relative to other body fluids and its detection is indicative of the presence of this body fluid. This test takes advantage of the amylase-catalyzed starch hydrolysis that results in short polysaccharides unable to react with iodine which is detected as a 'clearing zone' around sample wells containing amylase.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p 184-187.

Auvdel, Michael J. *Amylase Levels in Semen and Saliva Stains*, (1986) Journal of Forensic Sciences, 31 (2) 426-431.

Keating, S.M. and Higgs, D.F. *The detection of amylase on swabs from sexual assault cases*, (1994) Journal of the Forensic Science Society, 34 : 89-93.

2.0 SCOPE:

To provide a presumptive screening test for the presence of saliva on evidentiary items.

3.0 EQUIPMENT/REAGENTS:

Agarose (Sigma Type I or equivalent)
Soluble Starch
Amylase Diffusion Buffer
Iodine Solution
Petri Dish

4.0 PROCEDURE:

4.1 Sample and control extracts (dH_2O is used for negative control) should be prepared in dH_2O as usual (See BI-116).

4.2 Prepare a 0.1% agarose/0.01% starch gel by dissolving 100mg of agarose and 10mg of soluble starch in 10mℓ of Amylase Diffusion Buffer. Pour the gel into a (~9cm) petri dish, allow it to solidify, and punch wells ~2 mm in diameter, and at least 3 cm apart, into the gel.

4.3 Fill wells (do not overfill) with sample extracts and controls.

4.4 Mark petri dish for orientation and document sample placement.

4.5 Cover petri dish and allow diffusion overnight at 37°C. May be placed in a humid chamber.

4.6 To develop, flood the petri dish with ~10mℓ of 1:100 dilution of the iodine solution (100µℓ/10mℓ dH_2O), let stand a few moments to develop the purple color, then pour it off the plate's surface.

4.7 Record the results by measuring the diameter of the clear circles. For reporting, see **5.1**.

5.0 COMMENTS:

5.1 Positive controls should include 1:100 and 1:500 dilutions of fresh saliva as well as neat saliva or an extract of a known saliva stain. If the clear zone of an extract ≥ that of the 1:500 control, it is an indication of an elevated level of amylase in the extract and is reported as such. If an extract clears a zone smaller than the 1:500 control, there is an indication that amylase is present; however, there is no demonstration of an elevated level. An extract that demonstrates no clearing zone is reported as 'did not indicate the presence of amylase'.

5.2 Additional standards such as neat semen, neat urine or neat vaginal fluid may be tested as needed.

5.3 A negative result is not necessarily the total absence of saliva, and therefore, DNA testing should not be abandoned because of the absence of detectable amylase activity.

5.4 This test is not human specific, there may be reactive amylases in plants, bacteria, and non-human animals.

DETECTION OF URINE (UREASE)

1.0 BACKGROUND:

Urea, is a normal metabolite found in high concentration in urine. The urease reagent reacts with the urea present in a urine stain and releases ammonia, which may be detected with litmus paper.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p. 191-195.

Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 1978, Section 4.

2.0 SCOPE:

To provide a presumptive test for the presence of urine on relevant evidentiary material.

3.0 EQUIPMENT/REAGENTS:

Urease Reagent
Sterile/Nanopure H_2O
Small Corks (to fit 12x75mm test tubes)
12x75mm test tubes
Red Litmus Paper

4.0 PROCEDURE:

4.1 Cut out ~2.0cm^2 piece of suspected urine stain and controls, cut them into small pieces and place them into appropriately labeled 12x75mm test tubes.

4.2 Add 3-4 drops of dH_2O and 6-7 drops of Urease Reagent to each test tube.

4.3 Cut a slit into the bottom of each cork and insert a small piece of red litmus paper into the slit.

4.4 Place one of the corks (with litmus paper) into each tube; do not allow the litmus paper to come into contact with the liquid.

4.5 Incubate the tubes for 30 minutes at 37°C.

4.6 Note and document any change in the color of the litmus paper that occurs within the incubation time. A positive reaction (+) is recorded when the red litmus paper turns blue. When there is no color change noted, a negative(-) result is recorded.

5.0 **COMMENTS:**

5.1 Controls include positive (known urine stain) and negative (dH_2O blank) and a substrate control where appropriate and available.

5.2 The Urease Test is one of many presumptive tests for urine; a confirmatory test for the identification of urine in a dried stain is not available.

DETECTION OF URINE (CREATININE)

1.0 BACKGROUND:

Creatinine, the anhydride of creatine, is a normal constituent of urine and is a waste product of normal metabolism. It is present at high levels in urine compared to other body fluids. This test is based on its reaction with picric acid and is detected by a color change from yellow to orange.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p. 191-195.

Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 1978, Section 4.

2.0 SCOPE:

To provide a presumptive test for the presence of urine on relevant evidentiary material.

3.0 EQUIPMENT/REAGENTS:

Saturated Picric Acid Solution
5% (w/v) NaOH
Sterile/Nanopure H_20
Concentrated Glacial Acetic Acid
12x75mm test tubes

4.0 PROCEDURE:

4.1 Cut out ~0.5 cm^2 piece of suspected urine stain and controls and place them into appropriately labeled 12x75mm test tubes.

4.2 Add 0.5 mℓ of dH_20 to each test tube and extract for 15 minutes at RT.

4.3 Remove the substrate. Add 1 drop (~50 µℓ) of Picric Acid Solution and 1 drop (~50 µℓ) of 5% NaOH to each tube.

4.4 An orange color develops fully within 15 minutes and is stable for approximately 2 hours. The orange color is a positive indication of Creatinine. The negative control stain solution should remain yellow.

4.5 Document results in case notes. Record positive (+) or negatives (-). Analysts may use other descriptive word(s) (e.g., strong, weak,) or numerical grading (e.g., 1+ - 4+) as well.

5.0 COMMENTS:

5.1 Controls include positive (known urine stain) and negative (dH_2O blank) and a substrate control where appropriate and available.

5.2 This method is not specific for Creatinine. Although other chromagens are detected by this procedure, their concentrations are negligible.

5.3 Among other substances, glucose is reported to produce an orange color with alkaline picrate, although the color is pale. However, if there is likely to be confusion between this and a urine stain, the addition of 2 drops of glacial acetic acid renders a creatinine-containing sample pale yellow after a few minutes. (The color can be restored by adding a few drops of 5% NaOH). Heat is necessary to achieve the color change to pale yellow if the stain is glucose.

5.4 The Creatinine Test is one of many presumptive tests for urine; a confirmatory test for the identification of urine in a dried stain is not available.

DETECTION OF FECAL MATERIAL (UROBILINOGEN)

1.0 BACKGROUND:

Edelman's Test is a presumptive test for the presence of fecal material and is based on the detection of urobilinogen which is found in high concentration in feces. Urobilinogen, which is oxidized to urobilin, is soluble in alcohol and, in the presence of neutral alcoholic salts, will form a green fluorescent complex with zinc.

Gaensslen, R. *Sourcebook in Forensic Serology, Immunology, and Biochemistry*. (1983) U.S. Dept. of Justice, Washington, D.C., p. 191-195.

Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 1978, Section 4.

2.0 SCOPE:

To provide a presumptive test for the presence of feces on relevant evidentiary material.

3.0 EQUIPMENT/REAGENTS:

10% (w/v) Mercuric Chloride Solution
10% (w/v) Zinc Chloride Solution
Amyl (Isopentyl) Alcohol
Sterile/Nanopure H_2O
12x75mm test tubes
Alternate Light Source

4.0 PROCEDURE:

4.1 Cut out ~0.5 cm^2 piece of suspected fecal stain and controls and place them into appropriately labeled 12x75mm test tubes.

4.2 Extract samples in ~3 drops of dH_2O for 15-30 minutes at RT.

4.3 Remove the material and add ~3 drops of 10% Zinc Chloride Solution to the extract.

4.4 Add 5 drops of Amyl Alcohol to the extract and vortex.

4.5 Spin sample for 5 minutes on low (~2000 rpm) in the serological centrifuge and transfer the upper phase to a new 12x75mm tube.

4.6 To the upper phase, add 3 drops of 10% Mercuric Chloride Solution and observe any color change under both white and long wave UV light.

4.7 A positive reaction is recorded when green fluorescence is visible under long wave UV light. Absence of green fluorescence under long wave UV light is recorded as a negative reaction. Under white light, the solution may become rose-pink if urobilin is present.

5.0 COMMENTS:

5.1 Controls include positive (known fecal stain) and negative (dH_2O blank) and a substrate control where appropriate and available.

5.2 The Edelman's Urobilinogen Test is one of many presumptive tests for feces; there are no confirmatory tests available for the identification of fecal material.

5.3 The production of a green fluorescent complex is indicative of feces from humans and other carnivores. Due to the presence of chlorophyll, the feces of herbivores will produce an orange-pink fluorescence in this test. Test results giving this orange-pink fluorescence will be recorded as inconclusive.

EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS

1.0 BACKGROUND:

Many methods exist to obtain DNA, suitable for amplification, from a variety of sources. Caution must be exercised when selecting an appropriate extraction method, taking sample quantity into account.

Comey, CT et al. "DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis." J For Sci, Vol. 39, 1994, pp. 1254-1269.

Hochmeister, MN et al. "Typing of Deoxyribonucleic Acid (DNA) Extracted from Compact Bone from Human Remains." J For Sci, Vol. 36, 1991, pp. 1649-1661.

Hochmeister, MN et al. "PCR-based typing of DNA extracted from cigarette butts." Int J Leg Med, Vol. 104, 1991, pp. 229-233.

Yang, DY et al. "Technical Note: Improved DNA Extraction From Ancient Bones Using Silica-Based Spin Columns." Am J of Phys Anthropology, Vol 104:539-543, 1998, 539-543.

2.0 SCOPE:

To provide appropriate protocols for the extraction of DNA suitable for PCR amplification and subsequent analyses.

3.0 EQUIPMENT:

Qiagen BioRobot® EZ1
Qiagen EZ1 Tissue Kit and Forensic card
Centricon® Concentrator Devices
Microcentrifuge
15/50mℓ conical tubes
56/65°C heat block/oven
Fixed Angle Centrifuge
1.5mℓ microcentrifuge Tubes (1.5mℓ tubes)
MicroAmp Tubes
Coarse Sandpaper, Blender, Hammer, Chisel, Drill or Dremel

4.0 REAGENTS:

Stain Extraction Buffer (SEB)
PCR TE (TE, 10mM Tris-HCl; 0.1mM EDTA, pH 8.0)
Proteinase K (ProK, 20 mg/mL)
1M Dithiothreitol (DTT)
Phenol/Chloroform/Isoamyl Alcohol (PCIAA, 25:24:1)
Ethanol (EtOH)
Phosphate Buffered Saline (PBS)
Ethyl Ether
Xylene
10% SDS
FTA Purification Reagent
Chelex Reagent

5.0 DNA EXTRACTION PROCEDURES:

NOTE: Questioned and known reference samples must be extracted separately. If samples are extracted on the same day, questioned samples should be set up first.

The sample sizes listed below are the typical recommended amounts. Evidence samples vary in quantity and condition so samples sizes may be adjusted accordingly. The analyst should make an effort to retain sufficient sample for replicate testing if possible; however, those samples of limited size/quality may need to be consumed (See BI-QA 5.2.2).

Caution: See Comments 1.

5.1 EZ1 EXTRACTION OF WHOLE BLOOD SAMPLES:

Note: The BioRobot EZ1 may also be used for clean-up of samples that have already been extracted using the organic procedure to remove contaminants/inhibitors as needed. Transfer the extract to a EZ1 sample tube, bring the volume up to 200µℓ with Stain Extraction Buffer, and begin with step **5.1.2**.

5.1.1 Place ~3µℓ - 10µℓ whole blood into a EZ1 sample tube provided in the EZ1 DNA Tissue kit. Bring the volume up to 200µℓ with Stain Extraction Buffer.

5.1.2 Insert the Forensic Card into the card slot on the BioRobot EZ1 (if not already in place) and turn the instrument on. Note: the card may be left in place when the instrument is turned off.

5.1.3 Press "Start" to display the protocols menu. Press "2" for the "Trace" protocol.

5.1.4 Select the 200µℓ elution volume from the menu (option 3).

5.1.5 Press any key to proceed through the text displayed in the LCD, which guides you through the following steps to load the instrument.

5.1.6 Open the workstation door.

5.1.7 Examine the reagent cartridge(s) for the presence of precipitate. Invert each cartridge to mix the magnetic particles then tap the cartridge(s) to deposit the reagents to the bottom of their wells.

5.1.8 Insert the appropriate number of reagent cartridges (1-6 per extraction run) into the cartridge rack, snapping them into place. Additional samples (beyond 6) can be accommodated in subsequent instrument runs. Place the loaded cartridge rack into the instrument, followed by the tip rack.

5.1.9 Load 1-6 tip holders containing filter-tips into row 2 of the tip rack.

5.1.10 Load 1-6 opened and appropriately labeled elution tubes into row 1 of the tip rack. Make sure that the tube order matches that of the sample tubes.

5.1.11 Load 1-6 opened sample tubes from step 5.1.1 into row 4 of the tip rack.

5.1.12 Close the workstation door.

5.1.13 Press "Start" to start the extraction protocol.

5.1.14 When the protocol ends, the LCD displays "Protocol finished." To run another protocol, press "ESC" to return to the protocols menu. Otherwise, press "Stop" twice to return to the first screen of the LCD.

5.1.15 Open the workstation door. Remove and cap the elution tubes containing the purified DNA. Discard the cartridges, tip holders/tips, and sample tubes.

5.1.16 At the completion of all runs for the day, clean the piercing tool (Protocol #3 on the LCD), D-rings and tip adaptor, tip rack, cartridge rack, and interior of the instrument with 70% Ethanol, followed by nanopure water.

5.1.17 Switch off the instrument, leaving the Forensic card in place.

5.1.18 Proceed to realtime PCR (see BI-207) for quantification of the purified DNA obtained in step 5.1.15.

5.2 EZ1 EXTRACTION OF BLOOD/SALIVA/NON-SEMEN (TISSUE, EPITHELIAL CELLS) SAMPLES:

5.2.1 Place one of the following samples into a EZ1 sample tube provided in the EZ1 DNA Tissue kit: ~3mm^2 - 1cm^2 cutting/portion or swabbing of samples on cloth or porous materials (includes cigarette butts and envelope flaps/stamps), ~1/8 - 1/2 (~equivalent of previous sample size)cutting/portion of cotton swabs containing sample (samples deposited on non-porous objects may need to be collected onto a swab with a small amount of sterile deionized water, TE or SEB and the swab cut for testing), or ~3mm^2 - 5mm^2 portion of tissue.

5.2.2 Add the following to the tube:

190µℓ SEB
10µℓ Pro K

5.2.3 Mix and incubate at 56°C for a minimum of 20 minutes, up to overnight.

5.2.4 Remove the cuttings/substrate (if applicable) by piggyback/spin basket centrifugation at low speed (3,000 - 5,000 rpm) for 3-5 minutes and discard.

5.2.5 Insert the Forensic Card into the card slot on the BioRobot EZ1 (if not already in place) and turn the instrument on. Note: the card may be left in place when the instrument is turned off.

5.2.6 Press "Start" to display the protocols menu. Press "2" for the "Trace" protocol.

5.2.7 Select either the 50µℓ or the 200µℓ elution volume from the menu (option 1 or 3, respectively). The 50µℓ elution may be preferable for dilute samples or those suspected to be of low DNA concentration.

5.2.8 Press any key to proceed through the text displayed in the LCD, which guides you through the following steps to load the instrument.

5.2.9 Open the workstation door.

5.2.10 Examine the reagent cartridge(s) for the presence of precipitate. Invert each cartridge to mix the magnetic particles then tap the cartridge(s) to deposit the reagents to the bottom of their wells.

5.2.11 Insert the appropriate number of reagent cartridges (1-6 per extraction run) into the cartridge rack, snapping them into place. Additional samples (beyond 6) can be accommodated in subsequent instrument runs. Place the loaded cartridge rack into the instrument, followed by the tip rack.

5.2.12 Load 1-6 tip holders containing filter-tips into row 2 of the tip rack.

5.2.13 Load 1-6 opened and appropriately labeled elution tubes into row 1 of the tip rack. Make sure that the tube order matches that of the sample tubes.

5.2.14 Load 1-6 opened sample tubes from step 5.1.1 into row 4 of the tip rack.

5.2.15 Close the workstation door.

5.2.16 Press "Start" to start the extraction protocol.

5.2.17 When the protocol ends, the LCD displays "Protocol finished." To run another protocol, press "ESC" to return to the protocols menu. Otherwise, press "Stop" twice to return to the first screen of the LCD.

5.2.18 Open the workstation door. Remove and cap the elution tubes containing the purified DNA. Discard the cartridges, tip holders/tips, and sample tubes.

5.2.19 At the completion of all runs for the day, clean the piercing tool (Protocol #3 on the LCD), D-rings and tip adaptor, tip rack, cartridge rack, and interior of the instrument with 70% Ethanol, followed by nanopure water.

5.2.20 Switch off the instrument, leaving the Forensic card in place.

5.2.21 Proceed to realtime PCR (see BI-207) for quantification of the purified DNA obtained in step 5.2.18.

5.3 ORGANIC EXTRACTION OF BLOOD/SALIVA/NON-SEMEN (TISSUE, EPITHELIAL CELLS) SAMPLES:

5.3.1 Place one of the following samples into a sterile 1.5mℓ tube: ~3mm^2 - 1cm^2 cutting/portion or swabbing of samples on cloth or porous materials (includes cigarette butts and envelope flaps/stamps), ~1/8 - 1/2 (~equivalent of previous sample size) cutting/portion of cotton swabs containing sample (samples deposited on non-porous objects may need to be collected onto a swab with a small amount of sterile deionized water, TE or SEB and the swab cut for testing), ~3mm^2 - 1cm^2 portion of tissue, or ~10µℓ - 50µℓ whole blood.

5.3.1a Envelope Flaps/Stamps: Presoak the envelope flap/stamp cutting (steam may be used to loosen the seal prior to extraction) in 1.0mℓ of sterile water at 4°C for a minimum of 5 hours (may be left overnight). Remove the substrate by piggyback/spin basket centrifugation and discard. Remove all but 50µℓ of the supernatant and discard it. Proceed to **5.3.2** with the remaining pellet.

5.3.1b Optional (see **Comments 3**): Presoak bloodstains using 1mℓ of sterile PBS in a sterile 1.5mℓ tube. Vortex briefly, and incubate 30 minutes at RT. Vortex briefly, then spin at high speed in a microcentrifuge for ~1 minute. Using a micropipette, remove supernatant and proceed to **5.3.2**.

5.3.2 Add the following to the tube:

500µℓ SEB
15µℓ Pro K

5.3.3 Mix and incubate at 56°C for a minimum of 1 hour (may be left overnight). It is recommended that non-reference samples incubate for at least 3 hours when possible.

5.3.3a Optional: For most stains the cuttings/substrate will not interfere with organic extraction and need not be removed prior to proceeding to **5.3.4**. Larger cuttings/samples can be removed by piggyback/spin basket centrifugation at low speed (3,000 - 5,000 rpm) for 3-5 minutes and discarded. Proceed to **5.3.4**.

5.3.4 In a fume hood, add 500µℓ phenol/chloroform/isoamyl alcohol (PCIAA) to the stain extract. Mix vigorously by hand to achieve a milky emulsion. Spin in microcentrifuge for 3-5 minutes to achieve layer separation.

5.3.5 If the aqueous phase is clear, proceed to **6.0**. If it is not clear (e.g. cloudy or large or 'dirty' interface), transfer the aqueous layer to a fresh sterile 1.5mℓ tube. Repeat **5.3.4** 1-2 times until the interface is clean and aqueous phase is clear. Proceed to **6.0**.

5.4 DIFFERENTIAL EXTRACTION OF SEMEN-CONTAINING SAMPLES:

Note: For removal of sample from mounted slide, see **7.0**.

5.4.1 Place cutting/sample (the size of sample used will be case dependent and based upon microscopic exam and total sample amount) into a sterile 1.5mℓ tube and add ~150µℓ PBS or sterile deionized water. Agitate the substrate to loosen cellular material and place at 4°C for 1-4 hours (up to overnight).

5.4.2 Sonicate samples for ~20 minutes to loosen cellular material from the substrate and perform piggyback/spin basket centrifugation for 3-5 minutes. Without disturbing the pellet, remove all but ~50µℓ of the supernatant and discard.

5.4.2a Optional: Resuspend the pellet in the remaining 50µℓ and place 3-5µℓ on a slide for microscopic evaluation (See BI-118; BI-119). The substrate may be discarded if the pellet contains a sufficient number of spermatozoa; however, it may be desirable to add the substrate back to increase the total amount of DNA in the sample.

5.4.3 To the remaining cell pellet and substrate (if present) add the following:

500µℓ SEB
15µℓ Pro K

5.4.4 Mix and incubate at 56°C for 45 minutes to a maximum of 1 hour.

5.4.5 Label a new sterile 1.5mℓ tube. Remove substrate (if present) by using piggyback/spin basket centrifugation and discard. A final centifugation on high speed for ≥1 minute should be performed to further solidify the pellet.

5.4.6 Remove all but ~50µℓ of the supernatant, taking care not to disrupt the cell pellet in the bottom of the tube. Transfer this supernatant (epithelial cell fraction) to the new, labeled sterile tube and store at 4°C or proceed directly to **5.4.11**.

5.4.6a Optional: The purpose of a differential extraction is, typically, to obtain a sperm fraction that is void of any epithelial contribution. In instances in which there is an overwhelming proportion of epithelial cells to sperm that appear intact microscopically, steps **5.4.3-5.4.4** may, at the scientist's discretion, be repeated 1-2 times prior to proceeding to **5.4.7.** These additional supernatants do not need to be retained.

5.4.7 Wash the sperm pellet as follows: Resuspend the pellet in 1000µℓ PBS by vortexing briefly. Spin in a microcentrifuge for ~5 minutes at maximum speed (>10,000rpm). Remove all but ~50µℓ of the supernatant and discard it.

5.4.8 Repeat **5.4.7** 1-5 more time(s). In instances of low sperm amounts, additional washes are recommended. The final wash performed is to be done using 1000µℓ sterile deionized water.

5.4.8a Optional: Resuspend the pellet in the remaining 50µℓ and place 3-5µℓ on a slide for microscopic evaluation (See BI-118; BI-119). If intact epithelial cells remain, the pellet should be redigested (**5.4.3 - 5.4.8**).

5.4.9 To the remaining sperm pellet solution add:

500µℓ SEB
20µℓ 1M DTT
15µℓ ProK

5.4.10 Mix and incubate at 56°C for a minimum of 2 hours (may be left overnight).

5.4.11 In a fume hood, add 500µℓ phenol/chloroform/isoamyl alcohol (PCIAA) to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in microcentrifuge for 3-5 minutes to achieve layer separation.

5.4.12 If the aqueous phase is clear, proceed to **6.0**. If it is not clear (e.g. cloudy or large or 'dirty' interface), transfer the aqueous layer to a fresh sterile 1.5mℓ tube. Repeat **5.4.11** 1-2 times until the interface is clean and aqueous phase is clear. Proceed to **6.0**.

5.5 EXTRACTION FROM HAIR:

Note: For removal of hair(s) mounted on a slide, see **7.0**.

5.5.1 Examine the hair(s) under a stereomicroscope and note if there is the presence of cellular material at the root and the presence of any body fluid (e.g., blood or semen)or other visible contaminants on the hair shaft.

5.5.2 Once a suitable hair(s), preferably anagen, has been identified it may be washed to reduce surface dirt and contaminants. This may be accomplished by immersing the hair(s) in sterile, deionized water and gently swirling. Each hair to be analyzed should be washed separately in fresh water. Alternatively, the hair(s) may be placed in a 1.5mℓ tube containing 1mℓ 10% SDS and sonicated briefly. Again, each hair to be analyzed should be treated separately. If the presence of any body fluid is noted on the hair shaft, it may be removed for separate DNA analysis, if necessary, by soaking the hair in a minimal amount of sterile deionized water or PCR TE for 30 minutes. Process this extract as you would a bloodstain (see **5.3.1 or 5.1.1**).

5.5.3 Even if the hair(s) was washed prior to proceeding to **5.5.4**, it may still have cellular material on its surface that did not originate from the hair donor. Therefore, in addition to cutting off ~0.5 - 1.0cm of the root-end, a 0.5 - 1.0cm cutting of the shaft adjacent to the root may be processed separately as a control. The remaining shaft may be retained for subsequent analyses (e.g., microscopic exam, mitochondrial DNA).

5.5.4 To a 1.5mℓ tube, containing the hair sample, Add:

500µℓ SEB
20µℓ 1M DTT
15µℓ ProK

Mix and incubate at 56°C for minimum of 6-8 hours (may be left overnight).

5.5.5 In a fume hood, add 500µℓ PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in microcentrifuge for 3-5 minutes at high speed to achieve layer separation.

5.5.6 If the aqueous phase is clear, proceed to **6.0**. If it is not clear (e.g. cloudy or large or 'dirty' interface), transfer the aqueous layer to a fresh sterile 1.5mℓ tube. Repeat **5.5.5** 1-2 times until the interface is clean and aqueous phase is clear. Proceed to **6.0**.

5.6 EXTRACTION FROM BONES AND TEETH:

5.6.1 Obtain a fragment of bone and remove any tissue present, using ethyl ether (shake vigorously with a few mℓs of ether in a 15mℓ polypropylene tube) or by boiling briefly. For older bones, or those without adhering tissue, clean the outer surface by sanding. For teeth, begin with step **5.6.2**.

5.6.2 Rinse the bone/tooth, in the same manner, with distilled water.

5.6.3 Similarly, rinse the bone/tooth with 95% ethanol. Finally, clean the bone/tooth with a sterile cotton swab soaked with ethanol to ensure it is free of dirt and/or other contaminants. Allow bone/tooth to air dry.

5.6.4 Crush bone/tooth into small pieces/powder with blender (a chisel or hammer may be used initially). Alternatively, a drill and bit may be used on large bones to create a fine powder. Transfer the powder and/or small pieces created to a 1.5mℓ tube.

5.6.5 To the tube, add:

500μℓ SEB
15μℓ ProK

Mix thoroughly and incubate at 56°C overnight.

5.6.6 In a fume hood, add 500μℓ PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in a microcentrifuge for 3-5 minutes to achieve layer separation. Transfer aqueous layer to new sterile tube. Repeat this step until the interface is clean. Proceed to **6.0**.

Note: For aged bones, it may be necessary to process multiple samples and combine the extracts prior to proceeding to quantification.

5.7 EXTRACTION FROM FTA/CODIS DATABASE SAMPLES:

Note: Since the DNA remains bound to the FTA card, regular, non-filter pipette tips may be used throughout and a single tip may be used for each reagent. A multi-channel pipettor may be used for larger sample batches.

Traditional organic extraction may also be used on FTA samples if necessary (typically non-database samples).

5.7.1 Remove a "punch" from the FTA card using a 1.2mm Harris punch (this is accomplished by placing punch firmly on card and twisting 1/2 turn clockwise and 1/2 turn counterclockwise). Eject sample(s) into microAmp tube(s).

5.7.2 Add 150µℓ FTA reagent to microAmp tube(s), mix and incubate at RT for ~5 minutes.

5.7.3 Remove and discard FTA reagent from sample(s) (using either vacuum with small pipette tip or by micropipette).

5.7.4 Repeat **5.7.2-5.7.3** twice.

5.7.5 Add 150µℓ TE to microamp tube(s), mix and incubate at RT for ~5 minutes.

5.7.6 Remove and discard TE from sample(s) (using either vacuum with small pipette tip or by micropipette).

5.7.7 Repeat **5.7.5-5.7.6** twice.

5.7.8 Make sure the punch is at the bottom of the microAmp tube(s), using a sterile pipette tip if necessary. Place tubes, uncovered in 65°C oven for ≥2 hours.

5.7.9 Proceed to PCR Amplification (see BI-208).

5.8 CHELEX EXTRACTION:

Note: Chelex may also be used for clean-up of samples that have already been extracted to remove contaminants/inhibitors as needed. Start with step **5.8.4.**

5.8.1 Place an ~3mm^2 cutting of a bloodstain, or 3µℓ whole blood into a sterile 1.5mℓ tube and add 1mℓ of sterile deionized water.

5.8.2 Incubate at RT for 15-30 minutes with occasional mixing or gentle vortexing.

5.8.3 Spin in microcentrifuge for 2-3 minutes. Remove all but 20-30µℓ of the supernatant and discard it. If the sample is a bloodstain, leave the substrate in the tube.

5.8.4 Using a wide bore pipette tip, or a tip with the end cut off, add 200µℓ freshly prepared 5% Chelex. Make sure the Chelex solution is well mixed before adding to the sample.

5.8.5 Incubate at 56°C for 15-30 minutes.

5.8.6 Vortex at high speed for 5-10 seconds.

5.8.7 Incubate in boiling water for 8 minutes.

5.8.8 Vortex at high speed for 5-10 seconds, followed by centrifugation at high speed (≥ 10,000 rpm) for 2-3 minutes. This extract may be taken directly to realtime PCR (see BI-207) for quantification of the DNA.

Note: Care must be taken to not disturb the Chelex resin when removing sample for subsequent procedures. After storage and prior to sample removal, repeat step **5.8.8.**

6.0 DNA ISOLATION PROCEDURE:

Note: Centricon concentration of samples with high DNA concentrations will be performed separately from those with low DNA concentrations.

6.1 ISOLATION VIA CENTRICON CONCENTRATOR DEVICE:

6.1.1 Assemble a Centricon-100 unit according to the manufacturer's directions and label the unit.

6.1.2 Add 1.5mℓ of TE to the upper Centricon-100 reservoir.

6.1.3 Add the entire aqueous layer (approximately 500µℓ) to the upper reservoir containing TE. Discard the phenol mixture (including substrate if present) into the organic waste container in the hood. Discard the tube into a biohazard waste container.

6.1.4 Cover the Centricon tube with the retentate cup. Spin in a fixed angle centrifuge at ~3500 rpm for 10-20 minutes. The DNA sample will be concentrated in ~20-40µℓ of TE in the upper Centricon reservoir, while molecules with molecular weights of less than ~100,000 daltons will pass through the filter.

Note: The Centricon units are sensitive to rotor forces. Do not centrifuge above 2000 x g. Centrifugation time can be increased if the volume does not reduce to ≤40µℓ in the specified time.

6.1.5 Add 2mℓ of PCR TE to the concentrated DNA solution in the upper Centricon reservoir and repeat the centrifugation step as in **6.1.4.** Discard the effluent that has collected in the lower reservoir.

6.1.6 Repeat **6.1.5** for a total of 3 washes.

6.1.7 Invert the upper reservoir onto the retentate cup provided with the unit. Centrifuge at ~2500 rpm for 2 minutes to transfer the DNA concentrate into the cup.

6.1.8 Estimate the volume of the concentrate using a pipette to transfer to a labeled sterile 1.5mℓ tube. Proceed to realtime PCR (see BI-207) for quantification.

7.0 REMOVING MATERIAL FROM SLIDES:

7.1 FREEZING:

7.1.1 Place slide in -20°C freezer for 3-5 minutes.

7.1.2 Wearing safety glasses, pry the cover slip off.

7.1.3 Add a drop of xylene to dissolve the mounting medium.

7.1.4 Remove the hair and soak in 10-20mℓ xylene for 2-3 minutes to remove residual mounting medium.

Note: Sperm-containing slides are rinsed with sterile deionized water at this point and a suitable volume (~100µℓ) of stain extraction buffer (see **5.4.3**) may be added directly to the slide. Incubate ~5 minutes at RT and then by pipetting up and down, wash the sample off of the slide and transfer to 1.5mℓ tube. Repeat 3-4 times and proceed to **5.4.3.**

7.1.5 Rinse the hair briefly in absolute ethanol to remove the xylene and proceed to hair extraction under **5.5.**

7.2 SOAKING IN XYLENE:

7.2.1 Soak the slide in xylene for several hours until the cover slip can be slid or pried from the slide.

Note: This will likely remove markings from the slide.

7.2.2 Remove the hair and soak in about 10-20mℓ xylene to remove the residual mounting medium.

7.2.3 Rinse the hair briefly in absolute ethanol to remove xylene and proceed to hair extraction under **5.5**.

8.0 DNA EXTRACTS:

8.1 After a sample has been extracted and during subsequent analyses (i.e. quantification and amplification), the DNA extract may be stored at 4°C. For longer storage periods, the extract should be frozen at approximately -20°C. These extracts are in-progress work product during this stage(s).

8.2 Any extract remaining, following the completion of analysis will be retained in the corresponding case DNA packet (See BI-102).

9.0 Comments:

9.1 These methods employ the use of phenol that is a strong organic acid and may cause severe burns in addition to other effects. All operations with these chemicals should be performed in the hood with appropriate protective gear (gloves, lab coat and eyes protected by hood shield and/or goggles).

9.2 An appropriate reagent blank (for each type of extraction) should be carried through all extraction steps to check the purity of the reagents being used. There need only be one reagent blank per extraction run, it is not necessary to have a separate one for each case that is extracted at the same time.

9.3 Presoaking bloodstains with PBS may help to prevent inhibition of amplification by heme products, particularly when analyzing DNA obtained from samples of "heavy" bloodstains (e.g. control bloodstains).

9.4 These procedures represent the 'usual' protocol for a given material, however, any of these different extraction methods are suitable for all biological materials, though minor modifications may be necessary.

BI-207

DNA QUANTIFICATION: REAL-TIME PCR

1.0 BACKGROUND:

DNA methodologies that employ the PCR, such as STR analysis, necessitate consistent quantification of human DNA to obtain optimum data.

"Developmental Validation of the Quantifiler™ Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples," Green, R.L., et al, Journal of Forensic Science, Vol. 50, No. 4, pp. 809-825.

"Improving Efficiency of a Small Forensic DNA Laboratory: Validation of Robotic Assays and Evaluation of Microcapillary Array Device," Crouse, C., et al, Croat Med J 2005, Vol. 46, No. 4, pp. 563-577.

Quantifiler™ Kits (Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit) User's Manual, Applied Biosystems.

ABI Prism® 7000 Sequence Detection System User's Guide, Applied Biosystems.

2.0 SCOPE:

To provide a reliable method for the consistent quantification of small amounts of human DNA isolated from forensic samples.

3.0 EQUIPMENT/REAGENTS:

ABI Prism 7000/Computer
ABI Prism 7000 SDS Software
Pipettors
Pipette Tips
Quantifiler™ Human Kit
PCR-TE
20 μg/mℓ Glycogen (optional)
96-well Reaction Plate
96-well Reaction Plate Base
Optical Adhesive Covers
Centrifuge (optional)
Compression Pad
Microcentrifuge Tubes

4.0 PROCEDURE:

4.1 PREPARATION OF DNA STANDARDS:

4.1.1 Label 8 sterile microfuge tubes A through H or 1-8.

4.1.2 Dispense 30μℓ (or adjusted amount according to the kit QC results: Form 419-QC) of PCR-TE into tube A (Std. 1) and 20μℓ of PCR-TE into tubes B-H (Std. 2-8).

4.1.3 Mix the Quantifiler Human DNA Standard thoroughly by vortexing 3-5 seconds. Transfer 10μℓ to tube A (Std. 1). Mix the dilution thoroughly.

4.1.4 Prepare Std. 2-8 via a serial dilution by mixing and subsequent 10μℓ transfers from tubes A through H. The dilution series consists of 50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068, and 0.023 ng/μℓ, respectively.

4.2 REACTION PREPARATION:

4.2.1 Determine the number of samples to be quantified (including, at minimum, 2 sets of DNA standards).

4.2.2 Fill out the 7000 Load Sheet (Form 206-BI) on the 'Plate Setup' tab of the Excel spreadsheet/template. Print a copy for the case record. Choose the 'Plate Document' tab and ensure the information is correct and corresponds to the Load Sheet information entered. Perform a 'Save As' of the Plate Document Worksheet to disc (i.e. USB drive) for subsequent transfer to the ABI 7000. The document must be saved as a .txt file.

4.2.3 Calculate the volume of reaction components needed, based upon the number of samples to be quantified and adding 2 or 3 reactions to compensate for loss and variability due to pipetting. The following are the volumes needed per reaction.

Quantifiler PCR Reaction Mix	12.5μℓ
Quantifiler Human Primer Mix	10.5μℓ

Note: The volume of reaction components necessary to prepare the Master Mix will be automatically calculated upon Load Sheet data entry.

4.2.4 Thaw the primer mix and vortex 3-5 seconds. Pulse-spin prior to opening the tube. Mix the PCR Reaction Mix by gently swirling the bottle prior to use.

4.2.5 Place a 96-well reaction plate into its base, being careful not to touch the top or individual wells. Do not place the plate directly onto the counter or any surface other than its base or the ABI 7000 thermal block.

4.2.6 Prepare the Master Mix by pipetting the required volumes of primer and reaction mixes into an appropriately sized microcentrifuge tube. Mix by vortexing 3-5 seconds, followed by a pulse-spin.

4.2.7 Carefully pipet 23μℓ of the PCR Master Mix into the bottom of each reaction well to be used. 'Blowing-out' the pipette is not recommended to avoid splashing and/or bubbles in the well.

4.2.8 Add 2μℓ of sample or standard to the appropriate reaction well, being careful to avoid bubbles as much as possible.

4.2.9 Seal the reaction plate with an Optical Adhesive Cover. Proceed to **4.3**.

4.3 RUNNING THE REACTION:

4.3.1 Turn on the 7000 computer and login with the appropriate user name and password. After the computer has completely started up, power on the 7000 instrument, allowing it to warm up at least ~30 seconds. Launch the ABI Prism 7000 SDS Software.

4.3.2 Place a compression pad on the reaction plate (over the Optical Adhesive Cover) with the gray side down and with the holes placed directly over the reaction wells.

4.3.3 Open the instrument door by lifting the handle on the front and gently pushing the carriage back until it stops and locks into place. Place the plate (with compression pad in place) in the instrument thermal block so that well A1 is in the upper-left corner and the notched corner of the plate is in the upper-right corner.

4.3.4 Close the instrument door by gently pushing the carriage to release it and allowing it to slide forward into position over the reaction plate. Pull the handle down into place.

4.3.5 In the SDS software, select **File>New** and choose **Absolute Quantitation** for Assay, **96-Well Clear** for Container, and **Quantifiler Human** for Template.

4.3.6 Import the previously saved plate document by selecting **File>Import Sample Set-Up**. Browse to locate the saved .txt file and choose **OK**.

4.3.7 Review the plate document to ensure the appropriate detectors and tasks have been applied to each sample. Change the task for any unused wells to NTC in **View>Well Inspector**. Make any other changes, as necessary. Select the **Instrument** tab and review the thermal cycler conditions.

Note: Detectors are created during the initial instrument set-up and/or kit usage. Refer to the Quantifiler Kits User's Manual (page 2-11) for instructions on creating detectors if needed.

4.3.8 Save the plate document as a .sds file with the appropriate plate name.

4.3.9 Under the **Instrument** tab, click **Start** to begin the run. When the run has completed, proceed to **4.4**.

4.4 ANALYSIS AND RESULTS:

4.4.1 Open the plate document to be analyzed.

4.4.2 Select **Analysis>Analysis Settings** and verify the settings are set as follows: All for Detector, 0.200000 for Threshold, 6 for Baseline Start (cycle), and 15 for Baseline End (cycle). Click **OK**.

4.4.3 Select **Analysis>Analyze**.

4.4.4 In the **Results** tab, select the **Standard Curve** tab and choose Quantifiler Human as the detector. Review the data for inconsistencies from the following:

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

An R^2 value of <0.98 needs further analysis of the standard curve for problems. Refer to the Quantifiler Kits User's Manual (page 5-6) for troubleshooting guidelines.

The slope should fall within the typical slope range of -2.9 to -3.3. A slope of -3.3 indicates 100% amplification efficiency.

4.4.5 Select the **Amplification Plot** tab (in the Results tab) and choose either the Quantifiler Human, or the IPC detector. Ensure the Threshold is set to 0.20 before proceeding (**Note**: the threshold bar will be green if the data has been analyzed and red if analysis is needed). Highlight the sample(s) of interest in the table to view the associated plot(s). Review the plots for both detectors for amplification and/or inconsistencies.

4.4.6 Select the **Component** tab within the Results tab. View the Rox value listed. If the value begins approaching, or has fallen below 500, the instrument's halogen light bulb should be changed before proceeding with another run. See Comment 2.

4.4.7 In the Results tab, select the **Report** tab and highlight the sample(s) of interest to view the results. Review the Qty column to determine the amount of DNA present in each sample. Review the Internal Positive Control (IPC) C_T value for each sample. It should fall within a range of 20-30. If the value is <20 for a particular sample, there may be an indication of inhibition.

4.4.8 Export the report. Within the report tab, select **Tools>Report Settings** and check the appropriate boxes to be displayed in the report and click **OK.** Print a copy of the Standard Curve for the case record. Select **File>Export** to export the report (i.e. to USB drive) as a tab-delimited text file.

4.4.9 Open the 7000 Results Sheet (Form 209-BI) template in Excel. Import the tab-delimited text file into the **Raw Data** tab of the worksheet. Choose the **Results** tab and review the imported data. Delete any unused wells from the sheet. Adjust values in the Final concentration and ul Sample for Dilution columns. Print a copy of the results sheet for the case record. Perform a 'Save As' prior to exiting the template.

5.0 COMMENTS:

5.1 Refer to the Quantifiler Kits User's Manual for specific thermal cycler conditions, additional user information, and troubleshooting guidelines.

5.2 As Rox values approach 500, the Halogen Lamp may be tested to determine if replacement is needed. Place the Green Calibration Tray in the block. Select **File > New > Instrument > Calibrate**. Set Exposure time to **4096ms**, Lamp Control to **Max**, and select **Filter A**. Click Snapshot and observe results. Expected results should consist of red fluorescence displayed in all wells. Lack of fluorescence indicates the need for lamp replacement.

5.3 A system function test may be performed as needed. See the ABI 7000 User Manual (page 8-29) and/or the March 2006 User Bulletin for procedure. If a test fails, a service call should be placed.

5.4 In order to extend the life of the Halogen Lamp, the instrument should be turned off anytime it is not in use.

STR AMPLIFICATION: PP16

1.0 BACKGROUND:

DNA analyses have revolutionized forensic biology. The advent of PCR allowed scientists to analyze evidentiary material present in minute quantities and degraded states. The identification of forensically significant STR loci has allowed scientists to combine the discrimination attainable with the older RFLP technology with the speed and sampling capabilities of other PCR-based methodologies. The PowerPlex™ 16 allows the co-amplification of the core CODIS 13 loci, as well as, Amelogenin, and two pentanucleotide-repeat loci, Penta D and Penta E.

Butler, J. *Forensic DNA Typing: Biology and Technology Behind STR Markers.* (2001) Academic Press.

GenePrint® PowerPlex™ 16 System Technical Manual

2.0 SCOPE:

To provide a reliable method for consistent, high quality amplification of DNA from forensic and offender database samples ensuring the generation of suitable PCR product for capillary electrophoresis and analyses of these STR loci.

3.0 EQUIPMENT/REAGENTS:

BioHood
10% Bleach or Dispatch®
UV light
Thermocycler
Microcentrifuge
MicroAmp tubes
PowerPlex™ 16 Kit Contents
AmpliTaq Gold® DNA Polymerase

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

4.1.1 Based upon the quantity of DNA isolated and its initial concentration, the scientist should have all samples at an optimal concentration for amplification (e.g., 0.1ng/µℓ-0.4ng/µℓ). It is also convenient to have all samples that are to be amplified at the same time to be at the same concentration if possible for ease in the preparation of PCR Master Mix and reaction additions. For those samples that were deemed to be <1ng (or not detected at all), the maximum amplification volume (19.2µℓ for PowerPlex 16) should be used. For larger volume samples, it may be necessary to concentrate the sample prior to amplification. The analyst may also choose to extract, quantify, and combine additional sample prior to amplification as an alternative.

4.1.2 The amount of DNA template added to an amplification reaction should be targeted at ~0.5-1.0ng. For the positive control (9947A), 4-6ng template should be used with offender sample runs as the amplification cycle number is reduced for those samples.

4.2 AMPLIFICATION SET-UP:

4.2.1 Determine the number of samples to be amplified and label microAmp tubes (200µℓ) for identification. Label a microfuge tube(s) for the Master Mix. Place the labeled sample tubes in a rack or microAmp tray. The scientist may choose to irradiate the tubes with UV light at this point (≥ 15 minutes) while performing other preparations.

4.2.2 Thaw the Gold ST★R 10X Buffer and the PowerPlex™ 16 10X Primer Pair Mix.

4.2.3 Calculate the volume of reaction components needed based upon the number of samples (including extraction and amplification controls) to be amplified and adding 1 or 2 reactions to compensate for loss and variability due to pipetting. Use Form 210-BI for recording this information. The following is a list of the 'fixed' amounts to be added for a 25µℓ reaction.

Gold ST★R 10X Buffer	2.5µℓ
PowerPlex™ 16 Primer Mix	2.5µℓ
*AmpliTaq Gold®	0.8µℓ
[1]DNA Template + dH_2O	19.2µℓ

Note: The amount of Master Mix added to each sample is dependent on the volume needed to add the DNA template.

* AmpliTaq Gold® volume is based upon its typical concentration of 5U/µℓ. Check tube to verify concentration and adjust volume as necessary to add 4U of enzyme per reaction.

[1]For FTA/Offender database samples there is no volume for the DNA template so 19.2µℓ of dH2O will be added to these tubes.

4.2.4 Pipet PCR Master Mix into each reaction tube. The negative amplification control should be the last sample processed.

4.2.5 If DNA concentrations were not the same, add appropriate volume of dH_2O as necessary.

4.2.6 Pipet each DNA sample into the appropriate tube. Only the tube to which the DNA is being added should be opened at this time and only one DNA-containing tube should be open at any time (with the exception of the negative control which remains open throughout the process). Use 9947A control DNA for the positive amplification control and dH_2O for the negative amplification control. Again, making additions to the negative control last.

4.2.7 Ensure all of the sample tubes are closed tightly. Mix by finger or standard vortex and spin in microfuge, if necessary, to bring the reaction components to the bottom of the tube and remove any bubbles. Return samples to the rack or MicroAmp tray, placing them in position for the thermal cycler (record position on Form 210-BI).

4.2.8 Remove gloves and lab coat, placing gloves in biohazard container. Put on a new pair of gloves and, touching <u>only</u> the rack/MicroAmp tray, transport the samples to the thermal cycler in the Amp/PostAmp room, using the other hand on the door knob.

4.2.9 Place the samples into the thermal cycler. Do not set the rack down in this room. Remove gloves and return the rack to the biology lab. The rack may be placed in the hood under UV light for ~30 minutes at this time.

4.3 THERMAL CYCLING PARAMETERS:

4.3.1 After the samples have been placed in the thermal cycler, turn on the power and select the appropriate pre-programmed cycling profile.

4.3.1.1 For quantified DNA use 'pp16stdrun'; the cycling conditions are as follows:

95°C for 11 minutes, then:
96°C for 1 minute, then:

ramp 100% to:
94°C for 30 seconds,
ramp 29% to:
60°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **10 cycles**, then:
ramp 100% to:
90°C for 30 seconds
ramp 29% to:
60°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **20 cycles**, then

60°C for 45 minutes, then:

4°C soak

4.3.1.2 For non-quantified DNA (typically FTA/Offender database) use 'pp16buccal'; the cycling conditions are as follows:

95°C for 11 minutes, then:
96°C for 1 minute, then:

ramp 100% to:
94°C for 30 seconds,
ramp 29% to:
60°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **10 cycles**, then:

ramp 100% to:
90°C for 30 seconds
ramp 29% to:
60°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **17 cycles**, then

60°C for 45 minutes, then:

4°C soak

4.3.1.3 For additional cycles use 'pp16extra(3)'; the cycling conditions are as follows:

ramp 100% to:
90°C for 30 seconds
ramp 29% to:
60°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **3 cycles**, then

60°C for 45 minutes, then:

4°C soak

If, from the data generated on the Genetic Analyzer, it is determined that the signal for a FTA/Offender database sample falls below a 100-rfu threshold but in other respects appears to be good data, the scientist may remove 10μℓ of the PCR reaction, transfer it to a new microAmp tube and run the above cycling program. The negative control and reagent blank should be run through the same process. The positive control will likely exceed the maximum RFU threshold when taken through this process but the scientist may choose to perform the additional cycling and run a dilution on the 310 Genetic Analyzer as described in BI-210 4.4.2.4. See BI-210 4.4.2.5 RFU Threshold for additional information.

5.0 AMPLIFIED DNA PRODUCT:

5.1 After cycling has concluded remove samples from thermal cycler. Samples should be run on the Genetic Analyzer as soon as possible after amplification. Prior to capillary electrophoresis and/or before analysis is completed the samples may be stored at 4°C. For longer storage periods, samples should be frozen at -20°C. Amplified product is **ONLY** stored in the Amp/PostAmp room.

5.2 At a point in time after STR analysis is completed (i.e., case has been reviewed and report approved or CODIS data has been reviewed and approved for upload), the amplified product will be disposed of in a biohazard container in the amp/post-amp room. As needed, this container will be sealed and transported directly to the dishwashing room. The container will be placed into a second biohazard bag, sealed and disposed of with other biohazardous material.

6.0 COMMENTS:

6.1 Clean surfaces with freshly made 10% bleach solution or Dispatch® prior to set-up.

6.2 Wear gloves at all times during amplification set-up.

6.3 Mix all reagents thoroughly (e.g., vortex) and pulse-spin them in microfuge prior to dispensing.

6.4 A precipitate may form in the Gold ST★R 10X Buffer, this may be eliminated by briefly heating the solution at 37°C prior to mixing.

BI-210

STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS

1.0 BACKGROUND:

Any eukaryotic genome is interspersed with repeated DNA sequences that are typically classified by the length of the core repeat sequence, and the range of contiguous repeats typically seen or the overall length of the repeat region. STR (Short Tandem Repeat) loci are scattered throughout the genome occurring every 10,000 nucleotides or so, and have core repeat units of 2-6bp in length with overall lengths of less than 400 bp.

STR loci examined for human identification purposes were selected for the following characteristics: 1) high discriminating power (generally >0.9) with observed heterozygosity of >70%, 2) loci on separate chromosomes to avoid linkage, 3) ability to obtain robust, quality, reproducible data when multiplex amplification is performed, 4) low stutter, 5) low mutation rate and 6) small allele sizes (<500 bp) for enhancement of analysis of degraded samples.

By 1997, as the result of a community-wide forensic science effort, the following 13 STR loci, all tetranucleotide repeats, were selected as the basis for NDIS, the CODIS (COmbined DNA Index System) National Database: D3S1358, THO1, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, CSF1PO, vWA, D8S1179, TPOX, FGA. When all 13 CODIS core loci were examined, the average random match probability was found to be <1 in 1x10^{12} among unrelated individuals, offering the promise of individualization.

In addition to the 13 core CODIS loci, the PowerPlex™ 16 multiplex includes Amelogenin, a gender identification locus, and two pentanucleotide repeat STR loci, Penta D and Penta E. STR typing, with amplified products generated from this kit, separated by capillary electrophoresis on the 310 and/or 3130 Genetic Analyzer with data collection and analysis software employed in developing the genetic profiles, will be used to produce STR profiles from evidentiary material and convicted offender samples for entry into CODIS.

Butler, J. *Forensic DNA Typing: Biology and Technology Behind STR Markers.* (2001) Academic Press.GenePrint® PowerPlex™ 16 System Technical Manual

ABI PRISM™ 310 Genetic Analyzer User's Manual

ABI 3130/3130xl Genetic Analyzer Getting Started Guide

ABI 3130/3130xl Genetic Analyzer Mainenance Troubleshooting and Reference Guide

GeneMapper™ ID Software User Guide

2.0 **SCOPE**:

To provide a reliable method for generating STR genetic profiles from forensic casework and offender DNA database samples.

3.0 **EQUIPMENT/REAGENTS**:

310 and 3130 Genetic Analyzers with Data Collection Software
GeneMapper™ ID Software
Computers
Heating Block (or 9700 Thermal Cycler)
Benchtop Cooler
Capillaries
Capillary Arrays
Syringe
Sample Tubes and Septa
96 Well Reaction Plates and Septa
Buffer Jars and Septa
Buffer Reservoirs and Septa
POP4 Polymer
Genetic Analyzer Buffer
PowerPlex® 16 Kit Contents
PowerPlex® 16 Matrix Standards
Deionized Formamide
Nanopure Water

4.0 **PROCEDURE**:

4.1 AMPLIFIED FRAGMENT DETECTION USING THE 310

Note: Prior to using the ABI PRISM™ 310 Genetic Analyzer for samples, matrix standards must be run to achieve proper color separation of the dyes used for the amplification primers, allelic ladders and size standard. To prepare a matrix, four standards are run under the same capillary electrophoresis conditions that will be used for samples and allelic ladders. Use the 310 Matrix Standard set, which includes the Fluorescein Matrix, JOE Matrix, TMR Matrix and CXR Matrix for the blue, green, yellow and red matrix standards, respectively. This is performed when necessary due to performance, or after any instrument maintenance/repair that involves adjustment/replacement of the CCD camera or laser.

4.1.1 Turn on instrument, turn on computer and refer to ABI PRISM® 310 Genetic Analyzer's User's Manual for detailed instructions on instrument set-up. Shut down is performed in opposite order (computer, then 310). The computer may be shutdown after each run; the 310 should only be shut down if it will not be in use for extended periods. Fill-in appropriate information in the 310 Injection Log (Form 422A-QC).

4.1.2 Open the ABI PRISM® 310 Collection Software. In the manual control window, the scientist may use 'temperature set' to set the heat plate to 60°C so that it will be ready to run. Using File/New/Sample Sheet, create a 48-well Genescan® sample sheet as described in the ABI PRISM® 310 Genetic Analyzer's User's Manual. If there is room on the sample sheet, 'CCD' and 'SEQFILL' may be added (generally, as the last two samples). There is a 'Setup Check' sample sheet already on the instrument so that these samples may be placed in a pre-run by themselves, rather than adding them to the new sample sheet. Enter appropriate identifying information for other samples into the sample column as follows:

Matrix samples: FLUOR, JOE, TMR or CXR

Allelic Ladder: LADDER (or PP16 LADDER)

Controls: POS [or (+), etc.], NEG [or (-), etc.], BRB (blood reagent blank), RB (FTA reagent blank), MRB (male reagent blank), FRB (female reagent blank)

Case Samples: **XY99999999-(or /)ZZ...,**
(e.g., VM20010112-1AF or VM20010112/1AF) where:

X= Specimen Type (Q=Questioned; V=Victim; S=Suspect;

E=Elimination; M=Mother; F=Alleged Father; C=Child; FB=Paternal uncle; FS=Paternal Aunt; FM=Paternal Grandmother; FF=Paternal Grandfather, etc.)
Y = Letter for Lab (M, C or P)
999999999= Lab Case Number
ZZ...= numbers and letters that designate case Item (including 'M' for male and 'F' for female at end of number to delineate fraction).

CODIS samples: ID########## (e.g., ID2001001412).

4.1.3 Using File/New/Injection List, create a new Genescan® Injection List, selecting the appropriate sample sheet from the pull-down menu. Using pull-down selections, order samples, placing allelic ladders in the 1st and last injection positions as well as, at least every 20-25 samples in a long run. Move the 'CCD DUMMY' and 'SEQFILL DUMMY' to the 1st and 2nd injection positions, respectively if they were not run separately. Matrix samples are often analyzed in a separate run. However, they may be run with other samples, in which case they are run as contiguous samples either at the beginning or the end of a run.

4.1.4 Select a run module with the following settings:

GS STR POP4 (1mℓ) A
Inj. kV: 15.0
Run kV: 15.0
Run ˚C: 60
Run Time (minutes): 30
Matrix File: none
Autoanalyze: No

Inj.Secs: 3-10 secs

3 secs - Matrix Standards, Allelic Ladders and 1ng POS control DNA (injection times may be adjusted [3-10 seconds per analyst's discretion] but a 3 sec. inj. time for single-source samples estimated at ≥ 1ng generally produce good results).
5 sec.- Samples < 1ng generally produce good results.

4.1.5 To prepare samples for capillary electrophoresis:

Label sample tubes. For amplified products (including controls), typically 1µℓ-1.5µℓ rxn is added to 25µℓ of ILS Master Mix (made by adding 0.5µℓ ILS600 size standard/sample; 24.5µℓ deionized formamide/sample and adding quantities for N+2 in Master) that has been dispensed into sample tubes. For Allelic Ladders add 1µℓ Ladder to 25µℓ Master Mix. **Note:** The master mix may be altered by adding 1µℓ ILS600 size to 24µℓ deionized formamide if ILS peaks are too low.

Matrix samples (2µℓ) are added to 25µℓ of deionized formamide (without size standard).

4.1.6 Following sample addition, place septa on sample tubes, mix (spin as necessary) and heat denature for ~3 minutes at 95°C. Immediately chill in benchtop cooler (or on ice) for ≥3 minutes (perform on all sample types - ladders, matrix, controls and samples).

4.1.7 Assemble tubes for run into appropriate order (based on the sample sheet) in a 48-tube autosampler sample tray removing any moisture with a Kimwipe if necessary.

4.1.8 Place the autosampler tray in the instrument and close the doors.

4.1.9 Prior to hitting the 'Run' button to start the capillary electrophoresis, make sure that the autosampler has been calibrated if necessary, the syringe has sufficient polymer for the run and its current position is correct, and there are no bubbles that may interfere with the run. Click 'Run' and monitor electrophoresis in the 'Raw Data' and 'Status' windows. Each sample will take ~40 minutes.

4.1.10 If, at any point in the run, prior to the last injection, the scientist notices that a sample would benefit from re-injection (e.g., repeat because of bad injection or to vary injection times [from 3-10 seconds]) the scientist may insert a new row (Ctrl -I) and select that sample from the pull-down menu, changing the injection time if necessary.

4.1.11 After completion of the run finish filling out the 310 Injection Log (Form 422A-QC). Print Genescan® Injection List (~65%) for CODIS runs.

4.2 AMPLIFIED FRAGMENT DETECTION USING THE 3130

Note: Prior to using the ABI 3130 Genetic Analyzer for samples, a spectral calibration (matrix standards) must be run to achieve proper color separation of the dyes used for the amplification primers, allelic ladders and size standard. To prepare a matrix, four standards are run under the same capillary electrophoresis conditions that will be used for samples and allelic ladders. Use the 3130 Matrix Standard set, which includes the Fluorescein Matrix, JOE Matrix, TMR Matrix and CXR Matrix for the blue, green, yellow and red matrix standards, respectively. This is performed when necessary due to performance, or after any instrument maintenance/repair that involves adjustment/replacement of the CCD camera or laser.

Additionally, a Spatial Calibration must be performed prior to running any samples. The instrument uses images collected during the spatial calibration to establish a relationship between the signal emitted for each capillary, as well as the position where that signal falls and is detected by the CCD camera. This is performed any time a capillary is installed or replaced (including temporary removal of a capillary) or if the instrument is moved.

4.2.1 Turn on the computer, turn on the instrument, start Data Collection Software and wait for green squares to appear for all applications on the service console. Expand the necessary subfolders on the left tree pane of Data Collection. Refer to the ABI 3130/3130xl Genetic Analyzers Getting Started Guide for detailed instructions on instrument set-up (including creation of instrument protocols, results groups, and spatial calibration). Fill-in appropriate information in the 3130 Injection Log (Form 422B-QC).

Shut down is performed in the opposite order (Data Collection software, 3130, then computer). The Data Collection Software must be closed by choosing 'Stop All' and waiting for all red symbols to appear before closing. Never use the 'X' to close while green or yellow symbols are displayed.

4.2.2 Create a new plate record:

4.2.2.1 For a spectral calibration plate expand the tree pane of the Data Collection Software and click 'Plate Manager,' under 'ga3130xl'. Choose 'New', and fill in the dialog boxes, with 'Spectral Calibration' as the application. Fill in the applicable dialog boxes on the Spectral Calibration Editor as follows (clicking 'OK' when complete to save):

Sample Name: date_Spectral

Priority: May optionally be changed to a number <100 for injection priority.

Instrument Protocol 1: Choose the Spectral instrument protocol from the drop down menu

PowerPlex 16 specific run module and protocol settings for Spectral Calibrations are as follows:

Module Type:	Spectral
Template:	Spect36_POP4
Inj. kV:	3
Data Delay Time:	100
Run Time (seconds):	800

Protocol Type:	Spectral
DyeSet:	F
Array Length:	36
Chemistry:	Matrix Standard
Lower condition bound:	4.0
Upper condition bound:	12.0

Inj.Secs: 5

4.2.2.2 For a sample plate fill out the 3130 Load Sheet (Form 216-BI) on the '3130 Load Sheet' tab of the Excel spreadsheet/template. Print a copy for the case record or CODIS file. Choose the '3130 Plate Template' tab and ensure the information corresponds to the Load Sheet information entered. Verify the information on the template is as follows:

Container Type: 96-Well

Application Type: regular

GeneMapper: GeneMapper_Generic_Instance

Sample Name:

Allelic Ladder: LADDER (or PP16_LADDER)

Controls: POS [or (+), etc.], NEG [or (-), etc.], BRB (blood reagent blank), RB (FTA reagent blank), MRB (male reagent blank), FRB (female reagent blank)

Case Samples: **XY99999999_ZZ**..., where:

X= Specimen Type (Q=Questioned; V=Victim; S=Suspect; E=Elimination; M=Mother; F=Alleged Father; C=Child; FB=Paternal uncle; FS=Paternal Aunt; FM=Paternal Grandmother; FF=Paternal Grandfather, etc.)
Y = Letter for Lab (M, C or P)
99999999= Lab Case Number
ZZ...= numbers and letters that designate case Item (including 'M' for male and 'F' for female at end of number to delineate fraction).

(e.g., VM20010112_1AF)

CODIS samples: ID##########
(e.g., ID2001001412).

Priority: May optionally be changed to a number <100 for injection priority.

Sample Type: Sample Categories of 'Sample', 'Allelic Ladder', 'Positive Control', or 'Negative Control', may optionally be typed in.

Results Group 1: Enter the appropriate results group. These are typically denoted by the analyst initials and should automatically populate from the Load Sheet.

Instrument Protocol 1: Enter the appropriate instrument protocol (i.e., PP16_5 sec, PP16_3 sec, PP16_10 sec).

PowerPlex 16 specific run module and protocol settings for sample plates are as follows:

Module Type: Regular
Template: HIDFragmentAnalysis36_POP4
Inj. kV: 3
Run Time (seconds): 2000

Protocol Type: Regular
DyeSet: F

Inj.Secs: 3-10

3 secs - Allelic Ladders and 1ng POS control DNA (injection times may be adjusted [3-10 seconds per analyst's discretion] but a 3 sec. inj. time for single-source samples estimated at ≥ 1ng generally produce good results).

5 secs - Samples < 1ng generally produce good results.

4.2.2.3 Delete any unused wells. Perform a 'Save As' of the Plate Template Worksheet to disc (i.e. USB drive) for subsequent transfer to the ABI 3130. The document must be saved as a .txt file (tab delimited).

4.2.2.4 Import the previously saved plate record by selecting 'Import' on the 'Plate Manager' window. Browse to locate the saved .txt file and choose 'OK'.

4.2.2.5 Open the imported plate record by highlighting it and clicking 'Edit'. Review the information in the GeneMapper Plate Editor to ensure that it is correct or make changes as necessary. Click 'OK' when complete to save the plate record.

4.2.2.6 To perform more than one run of a sample (e.g. multiple injection times), select Edit/Add/Sample Run in the GeneMapper Plate Editor window. This will add additional Results Group and Instrument Protocol columns to the end of the plate record. These additional runs may be added at any point in the run, prior to the last injection, if the scientist notices that a sample would benefit from re-injection (e.g., repeat because of bad injection or to vary injection times [from 3-10 seconds]). Additional Results Groups and Instrument Protocols may also be filled in on the original Load Sheet template prior to importing.

4.2.3 In the manual control window, the scientist may choose to set the oven to 60°C so that it will be ready to run. Choose Oven in the 'Send Defined Command for' drop down menu box. In the 'Command Name' box, choose Turn On/Off oven, with a 'Value' of On, and click 'Send Command'. Next, in the 'Command Name' box, choose Set oven temperature, with a 'Value' of 60.0 and click 'Send command'. **Note**: once the oven has been turned on and the temperature set, the oven will only preheat for 45 minutes before shutting itself off.

4.2.4 Prepare samples for capillary electrophoresis:

4.2.4.1 For amplified products (including controls), typically 1μℓ-1.5μℓ rxn is added to 10μℓ of ILS Master Mix (made by adding 0.25μℓ ILS600 size standard/sample; 9.75μℓ deionized formamide/sample and adding quantities for N+2 in Master) that has been dispensed into the wells of a pre-labeled plate. For Allelic Ladders add 1μℓ Ladder to 10μℓ Master Mix. **Note**: The master mix may be altered by adding 0.5μℓ ILS600 size to 9.5μℓ deionized formamide if ILS peaks are too low.

4.2.4.2 Matrix samples are diluted 1:10 in Nanopure H_2O. 5μℓ of each matrix dye fragment is then added to 480μℓ of deionized formamide (without size standard). Load 25 μℓ of the fragment mix into each of four wells on the pre-labeled plate, which will include each of the four capillaries (e.g. wells A1 through D1).

4.2.5 Following sample addition, place a plate septa on the plate and heat denature for ~3 minutes at 95°C. Immediately chill in benchtop cooler (or on ice) for ≥3 minutes (perform on all sample types - ladders, matrix, controls and samples). **Note:** the plate septa may be cut to cover only those well columns being used on smaller plate runs.

4.2.6 Place the sample plate into the plate base and secure the plate retainer clip on top, making sure that no gray is visible through the holes.

4.2.7 Place the plate assembly in the instrument and close the doors. The plate map on the 'Plate View' window, under 'Run Scheduler' will turn yellow when the plate is in place and has been detected by the instrument.

4.2.8 Prior to running the plate, confirm that dye set F is selected and the correct active calibration for dye set F is set in spectral viewer.

4.2.9 Locate the plate record in the 'Plate View' window and highlight it by clicking on it once. With the plate record highlighted, click the plate map to link the plate to that specific record. The plate map will turn from yellow to green when it is successfully linked. Verify the correct scheduling of the run in the 'Run View' window. Select a run and confirm that the corresponding wells highlighted in the plate diagram are correct for that run. Make adjustments to the plate record if necessary.

4.2.10 Click the green Run Instrument arrow button in the toolbar to start the run. Monitor electrophoresis by observing the run, view, array, or capillaries viewer window. Each injection (set of four samples) will take ~45 minutes. **Note:** to run a duplicate plate record, the plate may need to be unlinked prior to linking the duplicated record. This is done by highlighting the currently linked plate record and clicking 'unlink'.

4.2.11 After completion of the spectral calibration run, open the 'Spectral Viewer' window to evaluate the spectral and set the active calibration. Confirm that Dye Set F is selected. Click on individual wells in the plate diagram to see results for each of the four capillaries. For each capillary, verify that four peaks are present in the spectral profile (upper pane), that the order of the peaks are, from left to right, blue-green-yellow-red, and that the peaks are regular in appearance. Next verify that four peaks are present in the raw data profile (lower pane), that the order of the peaks are, from left to right, red-yellow-green-blue, and that the peak heights are above 750RFU (1,000-4,000 RFU is ideal). If all four capillaries pass, then the calibration should be saved and set as the active calibration.

Note: All four capillaries must pass in order to accept a spectral calibration. A passing capillary will be colored green in the plate diagram. Additionally, capillary status may be viewed in the 'Event Log' under 'Instrument Status'. Rerun the spectral calibration as necessary until all four capillaries pass.

4.2.12 After completion of the run finish filling out the 3130 Injection Log (Form 422B-QC).

4.3 DATA ANALYSIS: GENEMAPPER® ID (GMID)

4.3.1 Data analysis is <u>NOT</u> performed on the instrument computers. Transfer the run folder (including the sample sheet for 310 runs and plate record for 3130 runs) to an analysis computer using a portable USB drive. After analysis and review are complete, a copy of the run folder and GMID project(s) will be stored on an analysis computer until CD/DVD archiving has been completed. The Run Folder on the instrument computer may be deleted at this point. Case-specific CDs will be made for discovery upon request.

Note: prior to data analysis, the appropriate panels and bins must be imported into GeneMapper® ID. Additionally, previously run 310 Macintosh data must first be converted to PC files using the 'Mac to Win' conversion program.

4.3.2 Set up the analysis methods for GMID analysis as follows (analysis methods are created and stored in the 'Analysis Methods' tab in 'GeneMapper Manager'):

'General' Tab: Name the analysis method so that it reflects what the method is (e.g. 310PP16-150RFU).

'Allele' Tab: Choose the appropriate bin set. Choose 'Use marker-specific stutter ratio if available', and ensure 'minus stutter distances' are from 3.25 to 4.75 for tetra and from 4.25 to 5.75 for penta. All others should be 0.

'Peak Detector' Tab: Advanced Peak Detection Algorithm, partial sizing (80-550 or 600), light smoothing, Local Southern size calling method with baseline window of 51 pts, min. peak half width = 2, polynomial degree = 3, peak window size = 15, and slope thresholds = 0.

Analysis range may be set to either full or partial and is empirically determined for each run and/or instrument. When using partial range, the start and stop points are determined by a review of the raw data and choosing points that will not include the primer peaks but will cover the size range of 80 to ≥500 bases.

Peak Amplitude Thresholds will depend on sample quality. Generally 150 rfu threshold in all colors. Rfu threshold may be raised in Blue, Green and Yellow for Allelic Ladder or Offender database samples only. Rfu threshold may be lowered to 50 rfu at the analyst's discretion (see **4.4.2** RFU Threshold). Lowering of rfu threshold below 70 rfu (to ≥50) should be done with caution and only if the data generally appears to be good, and without excessive baseline background or artifacts. Peaks below 50 rfu are deemed inconclusive.

'Peak Quality' Tab: The minimum peak height ratio for Heterozygote Balance should be set at 0.7 for casework samples and 0.5 for database (CODIS) samples. Set the max peak width to 1.5 bp and pull-up ratio to 0.05. The signal level and allele number may be set according to analyst preference and sample type.

'Quality Flags' Tab: The quality flags are only used as a tool to aid in data analysis and review (i.e. to assist in calling attention to potential artifacts or data quality concerns). These flag settings may be adjusted according to analyst preference and sample quality.

4.3.3 Create and store a size standard for GMID analysis, under the 'Size Standards' tab in 'GeneMapper Manager'. Name the size standard so that it reflects what the standard is (e.g. ILS600 80-600).

Data analysis will be performed using the 'Basic or Advanced' size standard. The size standard consists of the following peaks: 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 (the 80 and 600 peaks may be optionally defined by the analyst).

4.3.4 Create a matrix for GMID analysis of 310 data (3130 data do not require a matrix) under the 'Matrices' tab in 'GeneMapper Manager'. Matrix name is the date "MMDDYY" followed by "Matrix".

4.3.4.1 Review the Raw Data of the Fluor, JOE, TMR and CXR standards in the GMID project (see 4.3.5 and 4.3.6) and record an 'x' value that is after the primer peak, in an area of relatively flat baseline signal for each matrix standard. **Note**: the 'Analysis Range' must include ≥ 5 peaks for each matrix standard.

4.3.4.2 In the 'Matrix Editor' window, Click on a 'dye color' and select the corresponding Matrix Standard/.fsa file(e.g., Blue dye=FLUOR standard), and then enter the 'x' start value that you recorded from the Raw Data for that sample. Repeat for each of the Matrix Standards and click 'Create' to generate a new Matrix file.

4.3.4.3 Check to see that the numerical value trends indicate a good matrix (numbers on diagonal are '1.0000' and decrease from that value in each column.

4.3.4.4 Prior to clicking 'OK' to save the newly created matrix, it must be printed. This is done by pressing "shift" and "printscreen" simultaneously to take a snapshot of the matrix screen. Next, open Microsoft Word and paste the screen-shot into the blank document. The screen-shot may then be printed as a Word document. Once the matrix has been printed, click 'OK' on the matrix editor window.

4.3.4.5 Check Matrix quality by applying it to previously run samples, such as ladder, positive, and negative controls. Print each of the four, color plots and file in the QC log for the instrument.

The matrix may also be evaluated by applying it to the individual matrix samples in the GMID project. When applying it to itself, the Analysis Method chosen for analysis is as described in 4.3.2, except the peak detection algorithm must be Classic instead of Advanced. Examine the data generated. The samples should have peaks in the standard color but profiles should be relatively flat in the other 3 colors. With the exception of TMR (yellow) into CXR (red), bleed-through should not exceed 10%. If satisfied, print out a 4-color electropherogram plot, for each Matrix Standard. File in the QC log for the instrument.

4.3.5 Create a GeneMapper® ID Project:

4.3.5.1 From the GMID main menu, select File/Add Samples to project. Highlight the appropriate run folder in the pop-up window and click 'Add to List'. Once the run folder has been copied to the column on the right, click 'Add' to populate the project with the samples in the run folder.

4.3.5.2 In the Samples table, for each sample, select the sample type, analysis method, panel, size standard, and matrix (310 data only) from the pull-down lists. Ladders must be assigned the sample type of 'Allelic Ladder' for the analysis to occur. In order to use the control concordance quality flag, all controls must be marked appropriately as either 'Positive Control', or 'Negative Control'. All others may be marked as 'Sample'.

4.3.5.3 Save the project as the date MMDDYY, followed by Matrix, CODIS, or case # (and any other descriptors that may be necessary). A separate project should be created for individual cases. To do this, highlight the samples not associated with the particular case and choose Edit/Delete from the project main menu. Optionally, samples may be added to the project individually, rather than the entire run folder. **Note**: the analyzed project will be exported to the run folder at the completion of analysis/review.

4.3.5.4 Analyze the samples by clicking the green Analyze button. If the project has not already been saved, a prompt will appear to enter a project name before analysis will commence.

4.3.6 Evaluate GeneMapper® ID Data:

4.3.6.1 The Raw Data may be reviewed to determine analysis start/stop points, or to identify baseline problems, off-scale data, excessive 'spikes' or other anomalies that may interfere with data analysis and require re-injection or other corrective measures. Expand the run folder located in the navigation pane on the left. Highlight the sample(s) of interest to view the associated sample information, raw data and EPT data. Minimize or highlight the run folder to return to the main project window.

4.3.6.2 Check the 'SQ' (sizing quality) for all samples. A green square indicates that the sample has passed the sizing criteria and need not be manually examined. Examine the size standard of each sample with yellow and/or red 'SQ' to confirm correct assignment of fragment sizes. Highlight the sample(s) of interest and click the Size Match Editor button. If necessary, adjust the peak assignments by right clicking on a peak and deleting, adding, and/or changing values. If all peaks are correctly labeled but the quality score is below 1.0 (may be checked by choosing Tools/Check Sizing Quality), click the 'Override SQ' button to set the SQ to 1.0. Once all edits have been made, click 'OK' to save the changes and close the Size Match Editor (clicking 'Apply' saves the changes but leaves the Size Match Editor open). These samples are ready for reanalysis in the project window.

Note: Data may still be deemed acceptable without the ILS 60 and/or 600 bp peaks present. If additional peaks are assigned because of bleed-through of TMR peaks (typically Amelogenin peaks), the scientist may choose an Analysis Method, with an increased rfu threshold for the red channel to prevent these peaks from being detected, if desired.

4.3.6.3 Examine the blue, green, and yellow allelic ladders. Check that correct allelic assignments were made.

Note: GMID automatically averages all valid ladders in a run for genotyping. Genotypes are assigned by comparing the sizings of unknown alleles from samples with the sizings of known alleles contained within the averaged allelic ladders of each locus. A ladder(s) may be omitted from analysis by deleting it from the main project window prior to analysis.

4.3.6.4 Data may be examined in various combinations of colors and/or tables to identify bleed-through, spikes, stutter, -A, off-ladder variants, etc. Sample Plots viewed from the 'Samples' tab/window, allows all loci in a given color(s) to be viewed simultaneously. The Sample Plots view from the 'Genotypes' tab/window; however, allows loci to be viewed individually (more than one locus can be viewed at the same time by adjusting the number of panes displayed).

4.3.6.5 GeneMapper® ID includes a series of quality flags (PQVs) to alert the analyst of potential sample quality concerns. A green square indicates that sample data has passed all of the quality checks, but yellow or red indicate that the data has a problem with one or more of the quality checks. A yellow or red flag does not necessarily mean that the data is bad or unusable and the flags are not to be relied on solely. The analyst may choose to use the PQVs, in combination with manual data examination to aid in the identification of bleed-through, spikes, stutter, off-ladder variants, -A, etc. Once the data has been evaluated and deemed acceptable, the analyst may choose to override the yellow or red Genotype Quality (GQ) flag by right clicking on the flag in the Genotypes Sample Plots view. **Note**: overriding the GQ flag will cause all other flags to turn from the original color to gray.

4.3.6.6 All negative controls (including reagent blanks) should be examined to verify that each displays a relatively flat baseline in blue, green and yellow.

4.3.6.7 Review all samples (including positive controls) for the above listed 'artifacts' and evaluate: peak height and shape, matrix quality, and individual sample profiles. Compare each sample with the allelic ladder(s) and examine for off-ladder or microvariants, signals that were too low to be genotyped and assignment of genotypes to stutter peaks (or minor peaks that may have been subtracted as 'stutter', etc.

4.3.6.8 Reanalyze individual samples with different Analysis Methods, as necessary if the rfu cut-off will need to be changed.

4.3.6.9 Edit peaks as necessary, by right clicking on the peak label and selecting 'add allele', 'delete allele' or 'rename allele'. The allele should be labeled, at minimum with the allele call, however the analyst may select up to four allele labels, including peak height and size, from the 'Plot Settings Editor' window. **Note:** labels added to artifact peaks, such as spike, pull-up, etc. will appear in the Genotypes table as an additional allele.

4.3.6.10 Samples demonstrating an off-ladder (< or > smallest or largest ladder allele, respectively) or microvariant (alleles with incomplete repeats) allele(s) should be re-analyzed for verification where necessary (e.g., evidentiary profile in nonsuspect case, CODIS sample). Microvariants will be labeled and reported as "X.Y" (where X is the number of complete repeats and Y is the number of basepairs of the incomplete repeat). Off-ladder will be reported as > or < the largest or smallest ladder allele, respectively. **Note:** the nomenclature for upload to NDIS may necessitate a change in allele designation.

4.3.6.11 GMID automatically flags off-scale (camera saturation) data. This data may still be acceptable if it is limited to a few or a single peak and the overall data for that sample is of good quality (see 4.4.2.4).

4.3.6.12 Export an allele/genotypes table to Excel and save it in the run folder. The table will be printed for the case file or CODIS binder. The table may also be exported as a .cmf file (typically CODIS runs) for CODIS import. To create a .cmf file, the specimen category must be assigned and the export fields set in the 'CODIS Export Manager' under tools in the main menu.

4.3.6.13 Print the 'Samples Plots' for case files or the CODIS binder. Only one of the allelic ladders need be printed for documentation purposes.

4.4 STR INTERPRETATION GUIDELINES AND STATISTICAL ANALYSES

4.4.1 CONTROLS

4.4.1.1 The purpose of a **REAGENT BLANK** (RB) is to determine if the reagents used for DNA extraction/isolation were contaminated with human DNA and as a method for monitoring facility decontamination. In GeneMapper®, ID peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the ILS600 size standard. Electropherograms for the blue, green and yellow dyes should show a relatively flat baseline throughout the range (discounting primer signal, fluorescent 'spikes' or CXR bleed-through). If detectable signal, with characteristic 'peak' shape is visible in the electropherogram of a reagent blank and does not disappear upon re-injection, results for all associated samples may be deemed inconclusive (close examination at 50 rfu is performed on all samples to examine for presence of any alleles seen in the RB). Data may be deemed acceptable if contamination is 'isolated' to the RB. The reagent blank should be treated the same as the least concentrated DNA sample in terms of volume and amount amplified.

4.4.1.2 The purpose of the **POSITIVE AMPLIFICATION CONTROL** (9947A DNA supplied with the PP16 kit) is to assess the amplification process, ensuring that adequate sample amplified simultaneously would produce an appropriate signal. All expected alleles (see below) must be detected, using standard parameters or all of the samples associated with amplification may be deemed inconclusive. Data may be deemed acceptable if all alleles are present (though some are below 150-rfu threshold) **AND** the other positive control (Blind Control) appears as expected (i.e. the problem is confined to the 9947A sample).

LOCUS	**GENOTYPE**	**LOCUS**	**GENOTYPE**
D3S1358	14,15	THO1	8,9.3
D21S11	30,30	D18S51	15,19
Penta E	12,13	D5S818	11,11
D13S317	11,11	D7S820	10,11
D16S539	11,12	CSF1PO	10,12
Penta D	12,12	AMELOGENIN	X,X
vWA	17,18	D8S1179	13,13
TPOX	8,8	FGA	23,24

4.4.1.3 The purpose of the **NEGATIVE AMPLIFICATION CONTROL** is to determine if any human DNA contamination occurred in the process of amplification set-up (or beyond that point) and as another method of monitoring facility decontamination. In the GeneMapper® ID electropherograms, peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the ILS600 size standard. Electropherograms for the blue, green and yellow dyes should show a relatively flat baseline throughout the range (discounting primer signal, fluorescent 'spikes' or CXR bleed-through). If detectable signal, with characteristic 'peak' shape, is visible in the electropherogram of a negative amplification control and does not disappear upon re-injection, results for all of the samples associated with that amplification will be examined for the presence of the same peak(s). It is possible, since this control is processed last and its tube deliberately left open during the amplification set-up (to demonstrate maximum contamination potential), that it would be the only sample affected.

If extraneous peaks appear only in this control, the data for other samples associated with that amplification need not be deemed inconclusive. This occurrence should be documented and the scientist's determination (and basis for it) documented in the case file.

4.4.1.4 The purpose of a **BLIND CONTROL** sample is primarily to assess correct genotyping, however, it does take measure of all of the steps in the analytical process from extraction through allele designation. The blind controls consist of ~3mm^2 cuttings of previously typed bloodstains. Cuttings are prepared in batches and given random numbers. The scientist is not aware of the genotype of the sample. Source profiles are maintained by the unit supervisor/technical manager and are provided to the reviewer at the time of case review and only for the associated control(s). A blind control must be run with every batch of forensic cases (will generally be extracted with reference samples or non-semen evidence). The reviewing scientist will complete a Blind Control Check Form (Form 212-BI) for verifying correct genotype(s). A copy of this form will be included in each associated case file or CODIS Data Binder. **Note**: For CODIS offender buccal runs either an organic or EZ1 extraction or pre-extracted blind control (4-6ng) DNA may be used. Failure of the blind control, if isolated to that sample, will not deem other samples inconclusive.

4.4.2 RFU THRESHOLD:

4.4.2.1 For CODIS Offender database samples and reference blood or oral standards (excluding autopsy or other samples that may be degraded or of limited quantity), a minimum of 100 rfu should be achieved for data acceptance. If necessary, go back in the process as follows: repeat injection (changing injection time; 3-10 seconds allowable range), or perform re-analysis (i.e., changing amount of amplified product added for fragment analysis), or re-amplification (increase DNA template), or re-extraction.

4.4.2.2 For minor mixture components (or low-copy single-source forensic samples), a threshold of 50 rfus may be used (see **4.3.2** Peak Detection). However, depending on signal/baseline may be deemed inconclusive.

4.4.2.3 Peaks below the analysis threshold (based on data obtained and signal/baseline) will not be interpreted but should be noted as being present in the case notes (eg. on the table of results).

4.4.2.4 Peaks marked as off-scale in GeneMapper® ID (indicating camera saturation) will not be interpreted if multiple peaks are affected and if it causes excessive artifacts (i.e. split peaks, increased stutter, pull-up, etc.) which interfere with data interpretation (see **4.3.6.11**). If the overall quality of the data is not acceptable, the sample must be diluted, re-injected (3-10 seconds), reanalyzed (decrease the amount of amplified product added) or re-amplified (decrease DNA template) as deemed appropriate by the scientist.

4.4.2.5 The additional cycle (+3AMP) option may only be used on FTA/Offender database samples, and only when the following conditions are met: 1) the 100-rfu threshold has not been met but most alleles (≥ 70%) are ≥50 rfu and appear relatively balanced within a locus, 2) the sample has been extracted ≥2 times with similar results. Final data (following +3 AMP) must meet or exceed 100 rfus at all loci for Offender database samples.

4.4.2.6 Multiplex amplification kits are designed so that heterozygous loci in single-source samples generally demonstrate relatively balanced peak heights [typically ≥70% peak height ratio (phr)]. Some samples, although single-source, may at times demonstrate greater imbalance due to degradation, stochastic effects, primer binding site mutations, preferential amplification, etc. Peak height ratios for these loci (<70% phr for casework and <50% phr for CODIS samples) will be flagged in GeneMapper® ID.

4.4.3 EXTRA PEAKS (NON-MIXTURES)

4.4.3.1 PCR amplification of STR loci typically produces a minor product peak one core repeat unit shorter than the main allele peak (n-4 for tetranucleotide loci and n-5 for pentanucleotide loci). This minor peak is referred to as the **stutter** peak. Percent stutter generally increases with allele length and does not change significantly with the quantity of input DNA (peak heights within ~150-4500 RFU). The measurement of percent stutter may be unnaturally high for main peaks that are off-scale or due to problems with matrix performance and can be corrected by diluting (or reamplifying less DNA) the sample and/or applying a new matrix. Loci stutter values are listed in Appendix A to assess potential contribution to peaks in stutter positions.

4.4.3.2 Electronic or fluorescent **spikes** are random events that produce generally spike-shaped peaks in most or all dye colors at the same location (equivalent bp size) within a single injection. Peak heights usually vary between dye colors for a given spike. These anomalies are generally not reproducible and will typically be eliminated upon reinjection. If the spike is above the analysis threshod and falls within an allelic range that could interfere with either computer analysis or scientist's analysis, the scientist will label the spike in the GeneMapper® ID software so that it appears on the printed electropherograms.

4.4.3.3 Dye "**blobs**" are anomalies that typically occur in the same approximate location in multiple injections and do not always disappear upon reinjection. Blobs generally look like broad or irregular peaks and may occur in a single color or multiple colors at the same approximate location but can vary in height. The blob should be labeled on the electropherogram (in GMID) if it falls within a diagnostic region and is of significant size to potentially interfere with analysis.

4.4.3.4 **Bleed-through** or pull-up peaks are a result of the matrix not correcting for all of the spectral overlap (most common with the PowerPlex 16 kit from yellow into red) and may be increased due to off-scale peaks. These pull-up peaks are in the same location (same bp size) as peaks in another color(s) and are easily recognized. The presence of bleed-through should be labeled on the corresponding electropherogram (in GMID) if it falls within a diagnostic region and is of significant size to potentially interfere with analysis. If excessive bleed-through occurs in a color other than red, and is not due to off-scale data, a new matrix may be used at the analyst's discretion to correct for the problem.

4.4.3.5 *Taq* Polymerase can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3′ ends of double stranded PCR product, resulting in product one base pair longer than the actual target sequence (+A). Amplification parameters include a final extension time, so that the reaction is driven to full A addition (i.e. all product is +A). **Split-peaks** may occur as a result of incomplete A addition and appear as a single allele represented by two peaks one base pair apart (-A and +A). This can occur when the amount of template DNA is too great (overloaded sample). In this instance, *Taq* is unable to add the A nucleotide to the entire amount of product generated in the time allotted. These samples will typically contain off-scale data as well. Split peaks can be alleviated by incubating samples at 60°C for an additional 45 minutes, followed by dilution prior to reinjection. It may be necessary to re-amplify the sample with less template DNA.

4.4.4 MIXTURES

4.4.4.1 If, after the elimination of possible stutter and/or bleed-through, a profile shows at least 3 peaks at 2 or more loci, this is strong evidence of a mixture.

4.4.4.2 Loci that demonstrate only two alleles but have a heterozygous ratio of <70% may also be indicative of a mixture. However, if data are obtained from multiple loci, a scientist should expect to see this or other mixture indications (> 2 alleles) at additional loci.

4.4.4.3 Mixture assessment, in terms of determining the presence of a mixture (# of potential contributors) and probable locus genotypes is performed prior to examining the reference profiles.

4.4.4.4 In a probable 2-person mixture (no more than 4 alleles at any given locus) it may be possible to determine a major versus minor contributor at some or all loci. A major profile is one in which a distinct, predominant DNA profile is present (as determined by number of peaks, relative peak heights, and peak balance). A minor contributor is the less predominant DNA profile in the mixture.

4.4.4.5 For loci where distinct major/minor genotypes are discernible (this will occur rarely in a mix of more than two individuals' DNA), both genotypes may be reported.

4.4.4.6 Given that heterozygous peak ratios are not 100% (complete balance), caution must be exercised in determining "shared alleles", as a scientist does not know (*a priori*) which allele of a heterozygous individual may be predominant (i.e., the "highest rfu peak" in the 3-peak mixture may not be the shared allele). Calculations to determine the relative peak height ratios of 3-peak loci may be performed to assist in this determination (see Appendix B for calculation examples).

4.4.4.7 For samples where distinct genotypes are discernable, 'single-source' statistics are calculated for the individual profile(s) in the event of a profile match. It is more common; however, to only report a distinct major profile, due to the possibility of shared and/or dropped-out alleles in a minor component. Caution should be exercised when reporting a distinct minor profile for a sample.

4.4.4.8 Minor contributors in which a distinct minor genotype cannot clearly be determined will be reported as an inclusion/cannot be excluded (all minor alleles in the sample accounted for) or an exclusion (all or majority of the minor alleles in the sample not accounted for) and statistics will not be calculated for that minor contributor. It is possible that an individual may not be excluded as a possible contributor of the minor component, even if some of the reference alleles may not be present. This would occur with low level DNA and when there is an indication of possible allele drop-out.

4.4.4.9 Possible contributors to a mixture which distinct genotypes cannot be determined and/or mixtures of more than two individuals will be reported as inclusions (all reference sample alleles present in the mixture), cannot be excluded (majority of reference alleles present in the mixture but may be low level and have some indication of allele drop-out), or exclusions (majority or all of reference alleles not present in the mixture). Statistical interpretation will demonstrate the significance (or lack thereof) of the data.

4.4.4.10 A sample with interpretable peaks at one or more loci may be reported even if no peaks are detected at additional loci (i.e. partial profiles); statistical interpretation will demonstrate the significance (or lack thereof) of the data.

4.4.5 STRs: STATISTICAL GUIDELINES

To present the significance of a match between STR profiles, the scientist uses the population distribution (frequency) of alleles at the various loci examined to assess how likely it is that this match might occur by chance. This general concept forms the basis of all calculations used in the reporting of forensic "matches".

4.4.5.1 The frequency of occurrence of a STR profile obtained from an evidentiary sample will be determined by examination of the frequency in the FBI's Caucasian, African American and Hispanic databases. Calculations will be performed using the Popstats and/or DNAView programs. Additional population data may also be used when available and relevant to a particular case (See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies).

4.4.5.2 The frequency for a heterozygous profile is determined by the equation $f_{(pq)} = 2pq$.

4.4.5.3 The frequency for a homozygous profile is determined by the equation $f_{(pp)} = p^2 + p(1-p)\theta$, where $\theta = 0.01$ except where small isolated populations (e.g., Native Americans) may be relevant, in which case, $\theta=0.03$.

4.4.5.4 For single-source evidentiary samples (or mixtures for which a distinct genotype(s) is discernible) the statistical consideration will be in the form of a **RANDOM MATCH PROBABILITY** (RMP; or inverse probability of inclusion). The RMP is the inverse of the calculated profile frequency (e.g., for $f_{(\text{STR profile})} = 2 \times 10^{-14}$, RMP= 1 in 5×10^{13}; See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies).

4.4.5.5 For mixtures for which distinct genotypes are not discernible, and one or more of the associated reference samples are included in the mixture, the scientist may elect to use either the **LIKELIHOOD RATIO (LR)** or **PROBABILITY OF EXCLUSION (PE)**.

The LR compares the probability of the occurrence of the evidentiary profile under two hypotheses regarding the composition of the profile and is in the form:

$$\mathbf{LR} = \frac{P(\text{evidentiary STR profile}|H_1)}{P(\text{evidentiary STR profile}|H_2)}$$

The larger the **LR**, the more likely H_1 was the true hypothesis (See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies). For a paternity calculation, this corresponds to the PI (Paternity Index).

The PE (P_E) represents the probability that a randomly selected individual would possess one or more alleles inconsistent with the crime scene stain (or paternity). It is the complement of the RANDOM MAN NOT EXCLUDED (or "inclusion probability"; P_I).

The PE does not take into account the number of contributors, the principals' genotypes (i.e., the fact that they could account for the profile) or the evidence (e.g., peak height differences allowing probable donor assignment). It is calculated as follows:

$P_E = 1 - P_I$

Where $P_I = (p_1 + p_2 + p_3 \ldots p_x)^2$ (the square of the sum of the frequencies of all alleles present in the evidentiary sample).

The $P_{E\text{combined}}$ (for all of the loci combined) is as follows:

$P_{Ecombined} = 1 - [(1- P_{E1})(1- P_{E2})(1- P_{E3})...(1- P_{E15})]$ (See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies).

4.4.5.6

In addition to the LR and PE used in paternity, the probability of paternity may be used. However, given that this statistic requires non-genetic information (i.e., the prior odds of paternity), the prior odds used (e.g., 50%) should be explicitly stated (See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies).

4.4.5.7

In many forensic cases, the denominator of the RMP obtained for an evidentiary item, from the analysis of several polymorphic STR loci, exceeds the population of the world several-fold. However, no reasonable individual would make the assertion that every individual in the world need be considered a potential DNA source in the context of a given case. **'SOURCE ATTRIBUTION'** (see Budowle, B. et al, Source Attribution of a Forensic DNA profile. *Forensic Science Communications*. 2(3) July 2000) is the result of a statistical approach to 'operationally' define uniqueness (assess whether a given multi-locus DNA profile could be considered unique for a given case).

The equation $p_x \leq 1-(1-\alpha)^{1/N} \approx \alpha/N$, is used to determine maximum RMP (p_x) that would support 'source attribution' for a relevant population sample size (N) and selected confidence limit (i.e., $\alpha=0.01$; $1-\alpha=99\%$ confidence).

The FBI has selected an upper confidence limit (UCL) of 99% ($\alpha=0.01$) and an "N" equivalent to the U.S. population (2.6×10^8 pre-2000 census). This is reasonable as the FBI performs casework for jurisdictions all over the country and this calculation would provide a uniform approach to be used regardless of jurisdiction. For these figures, an RMP of $<3.9\times10^{-11}$ (or less than 1 in 2.6×10^{10}) would confer 99% confidence that the evidentiary profile is unique in the population. However, an additional 10-fold conservation factor, as recommended in NRC II, is added to this figure resulting in a frequency of less than 1 in 2.6×10^{11} for the reporting of source attribution.

In Idaho, using 2000 consensus figures, an operational population (N) of 1.6×10^{7} has been selected (representing the sum of the populations of Idaho and the six surrounding states: ID=1.3×10^{6}; MT=0.9×10^{6}; NV=2.0×10^{6}; OR=3.4×10^{6}; UT=2.2×10^{6}; WA=5.9×10^{6} and WY=4.9×10^{5}). Therefore, an RMP of less than 1 in 1.6×10^{10} (including 10-fold conservation) will define source attribution (at 99% UCL) for analyses performed in this laboratory (See **Biology QA Manual, section 11.2.5** for reporting of statistical frequencies).

5.0 Comments:

5.1 The 310 POP4 Polymer and the 3130 POP4 Polymer are different and are not to be used interchangeably.

5.2 The 3130 Data Collection Software does not allow the entry of spaces or dashes in titles, sample names, etc. An underscore must be used in place of spaces when entering information.

BI-301

CODIS SAMPLE RECEIPT AND DNA TRACKER ENTRY

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. It is important however, that samples entered into the database be given a unique identifier, which does not include any personal or identifying information, in order to maintain the confidentiality of the individual. Each laboratory must develop a method of identifier assignment so that each sample may be tracked, and identified at a later time, if the need arises.

Idaho Statutes: Title 19, Criminal Procedure, Chapter 55
"The Idaho DNA Database Act of 1996"

ISP Forensic Biology Quality/Procedure Manual, Appendix C

2.0 SCOPE:

To provide a method for tracking offender database samples submitted for STR testing and CODIS entry, while ensuring individual confidentiality.

3.0 EQUIPMENT/REAGENTS:

Computer Workstation with ISP Intranet Access
Barcode Equipment
Court Orders, Database Samples, and Report Forms

4.0 PROCEDURE:

4.1 SAMPLE RECEIPT:

4.1.1 Offender DNA samples and their corresponding DNA Collection Report Forms received by the laboratory are to be marked with the date of receipt and the initials of the scientist who received them. The sample and report form may be mailed to the laboratory separately; in the event that a sample has not been received, the submitting agency should be notified.

4.1.2 Where possible, compare the DNA sample card information to that of the Collection Report Form to ensure accuracy. Data for a sample may be entered in absence of a DNA Collection Report form. Additionally, an IDOC# is not necessary for data entry into DNA Tracker, but it is preferred. Contact necessary IDOC personnel or search the corrections website (www.corrections.state.id.us) for a number if one has not been recorded. The DNA Collection Report Form will be retained after DNA Tracker data entry.

4.1.3 The offense listed on the DNA sample card and/or Collection Report must be a qualifying offense under Idaho Code 19-5506 in order for the sample to be entered into DNA Tracker and subsequently CODIS/NDIS. The corrections website listed above and/or ILETS will be consulted for more information if the offense listed is non-qualifying or unclear. The scientist relies, in good faith, on the information provided by the submitting agency for entry into Tracker. It is not necessary, nor is it the scientist's responsibility to verify the offense(s) of every Offender, if the information provided by the submitting agency qualifies as listed.

4.1.4 Samples received in the laboratory that do not have an associated qualifying offense will not be entered into DNA Tracker. The collection report (if present) will be marked to indicate that the sample is a non-qualifying offense and returned to the submitting agency. If there is no collection report, a copy of the sample card will be made, marked in the same manner, and returned to the submitting agency. The sample(s) will then be destroyed.

4.2 COURT ORDER RECEIPT:

4.2.1 Court order forms received by the laboratory are to be marked with the date of receipt and the initials of the scientist who received them.

4.2.2 The offense listed on the Court order must be a qualifying offense under Idaho Code 19-5506 in order for the court order information to be entered into DNA Tracker.

4.2.3 If the offense on the court order is not a qualifying offense, no further action will be taken. The court order will be marked to indicate it is a non-qualifying offense and returned to the submitting agency.

4.3 DNA TRACKER PRE-ENTRY SEARCH:

4.3.1 Prior to data entry for any new sample or court order, a database search is performed to eliminate duplicate offender entry. *Log on* to the DNA Tracker database program, located under Forensics on the ISP Intranet.

4.3.2 A duplicate offender search will be performed using the 'Name' field, followed by at least one of the 'ID' fields to maximize the potential for locating an offender. Note: the 'DOB' field may only be searched in combination with a name.

4.3.3 If all of the searches return 'No matches found', the data for the new sample or court order may be entered as a 'New Offender' (see **4.4**).

4.3.4 If a record(s) is returned that meets the criteria, the data is examined and compared with the new sample or court order received. If it is determined that the Offender already exists in DNA Tracker, the new sample or court order and any additional Offender information will be entered under the appropriate tabs for the already existing Offender. Each sample received for an offender will be assigned a unique barcode number and will be retained in the laboratory (see **4.4**).

4.4 DNA TRACKER ENTRY:

4.4.1 Enter basic Offender information from the court order, sample card, and/or DNA Collection Report form as follows:

1) For Offenders not currently in Tracker, Click 'New Offender' at the top of the screen and fill in each of the appropriate fields with the Offender's primary information. Additional information for Offenders already in Tracker may be entered by clicking the 'Edit Basic Details' tab for that Offender.
2) Verify all of the information is correct and press the 'Save' button in the top right corner of the screen.
3) Add any additional, alias names, DOB's, SSN's, and State Identification Numbers on the appropriate alias tab(s) that become available after saving the new offender. Click the 'Save' button after each entry.

4.4.2 Enter each offense and its associated information from the court order, sample card, and/or DNA Collection Report form as follows:

1) Click the 'Offenses' tab for the saved offender.
2) Choose the appropriate offense from the pull down menu and enter the corresponding information into the remaining fields.
3) Verify the information is correct and press the 'Save' button in the top right corner of the window. Multiple offenses for the same Offender must be entered and saved individually.
4) Offense information may be updated/edited if additional data is received at a later time (e.g. when a sample arrives, fulfilling a court order or an additional sample for the same offense is received). Under the 'Offenses' tab, click on the appropriate offense code listed in the table of offenses. Enter the appropriate information and click the 'Save' button.

4.4.3 Enter Court Orders for an Offender as follows:

1) Click the 'Court Orders' tab for the saved offender.
2) Enter the court order issue and received dates. Note: the order received date will automatically populate in the sample history, once a sample has been received fulfilling that court order.
3) Click the 'Add Offense' link and choose the appropriate offense/court case from the pull down menu. Note: the offenses in the pull down list are those previously entered in the 'Offenses' tab. A court order must have an associated offense in order to be entered; therefore, the offense information must be entered prior to the court order.
4) Verify the information is correct and click the 'Save' button.
5) Once the court order has been entered it is placed in the appropriate filing cabinet located in the CODIS office.

4.4.4 Enter samples for an Offender and print sample barcodes as follows:

1) Click on the 'Samples' tab for the saved Offender.
2) Enter the sample information into the appropriate fields, leaving the barcode field blank (Tracker will automatically generate a unique barcode number if the field is not filled in).
3) If the submitting agency is not listed in the agency pull down menu, it may be added by clicking on the 'edit agencies' button to the right of the agency field.
4) If the sample received is pursuant to a previously entered court order, click on the 'Show Unfulfilled Court Orders' link. Click the box next to the appropriate court order to mark it as fulfilled by the sample being entered.
5) Enter the number of barcode labels to be printed. One label is to be placed on the DNA sample card/folder and one inserted behind the

FTA card envelope for placement on the FTA card at the time of analysis.

6) Verify the information is correct and click the 'Save' button. Barcode labels will automatically print upon saving the sample information.
7) Staple the DNA Collection Report (if present) to the inside of the DNA sample folder and attach the appropriate barcode labels. The labeled DNA sample card, with collection report will be placed in one of the filing cabinets located in the CODIS office.
8) Barcode labels may be printed at a later time for individual samples, by clicking on the appropriate sample from the 'Samples' tab or for multiple samples, by using the 'Batch Samples' function at the top of the screen.

BI-302

CODIS SAMPLE DATA ENTRY AND UPLOAD

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. Accurate data entry for upload to NDIS (National DNA Index System) is essential.

It is the responsibility of the Forensic Scientist to generate profiles from convicted offender and/or forensic samples, determine CODIS eligibility, review other scientist's profiles, to enter their profiles into CODIS and to notify the CODIS Manager of any potential problems associated with their CODIS DNA profiles. The CODIS Manager is responsible, in part, to serve as the laboratory's chief point of contact for all CODIS communications, maintain CODIS software updates and security, update/add/remove CODIS users, and upload profiles to NDIS.

DNA profiles entered into CODIS will be done so according to CODIS and NDIS Operational Procedures. Idaho currently allows for data entry into the following Indices: Offender (convicted offender); Unidentified Human Remains (deduced victim known, unidentified person); Missing Persons (alleged father/mother, biological child/father/mother, sibling, deduced victim known, missing person, maternal/paternal relative); Forensic Mixture; and Forensic (known and unknown). Profiles entered into the Forensic Index include both solved and unsolved cases in which the profile is associated with a crime and believed to be attributable to the putative perpetrator. Suspect reference samples and profiles matching the victim(s) and/or any elimination samples (e.g. consensual partner samples) may not be entered.

2.0 SCOPE:

To provide a mechanism to ensure accurate data entry for all offender and forensic database samples

3.0 EQUIPMENT/REAGENTS:

CODIS Computer Workstation

Allele Tables and/or CMF files generated from sample analyses.

CODIS Training Manual

4.0 PROCEDURE:

4.1 SAMPLE DATA VERIFICATION:

4.1.1 Genotypic data (allele calls) are checked for accuracy and verified during the CODIS/Casework Review process and documented on the appropriate form (Form 306-BI and Form 214-BI, respectively).

4.1.2 Forensic and Missing Person/Unidentified Human profiles are verified for CODIS eligibility and Index and documented on the CODIS entry form (Form 218-BI).

4.1.3 Additionally, when 'STR Data Entry' is used to enter individual sample data (generally forensic samples) verification of 1st and 2nd 'reader' is automatically achieved prior to NDIS upload.

4.2 SAMPLE DATA ENTRY IN CODIS:

4.2.1 Once sample data accuracy (forensic and offender) has been verified, data may be entered into the CODIS database either by use of the 'STR Data Entry' module to enter specimen ID/info and allele calls for individual samples or by using the 'Import' function for the entry of batches of data contained in a .cmf (or equivalent) file. Refer to the CODIS Training Manual and course documentation for specific steps in accomplishing these tasks.

4.2.2 A second 'read' must also be entered for the individual samples through 'STR Data Entry'. This may be done by the analyst entering the sample data, or by another analyst logging on and entering the allelic data in the second read box. If a single individual enters both reads, the entire profile (i.e. all loci) for the first read must be entered prior to entering any data into the second read. A 'check' indicates agreement between readers at individual loci and discrepancies in entry must be rectified before upload to NDIS.

4.2.3 When using the 'Import' function, the scientist will open the appropriate .cmf (or equivalent) batch file select 'validate import'. This will ensure that any typos or inconsistencies (i.e., variant allele definitions/equivalencies) will be identified prior to import and may be corrected. Once the batch file has been validated for import (corrections performed if necessary), 'import' is selected by the scientist and the process of importing the batch file data into SDIS will commence.

4.3 CODIS DATA UPLOAD:

4.3.1 NDIS There are various reasons that some samples present at SDIS should not be uploaded to NDIS. Prior to NDIS upload, these samples will be selected in Specimen Manager and 'unmarked for upload'. Generally speaking, an incremental upload will be performed. In Specimen Manager, 'incremental upload' is checked on the 'upload' pull-down menu and 'send upload' is selected. The upload is sent to NDIS as a message attachment via DNACOMM. If any 'candidate matches' are identified at NDIS, a match message will appear in DNACOMM and they will also be reflected in Match Manager. For hit verification see BI-303.

5.0 COMMENTS:

5.1 Refer to CODIS Training Manual and course documentation for more specifics if necessary.

5.2 The CODIS software is redundant and there is generally more than one way to accomplish many tasks. Using a mechanism other than that listed here is acceptable.

5.3 The CODIS software is updated periodically and any necessary changes in procedure provided with new updates supercedes those in procedures written prior to update, if appropriate.

5.4 The sample history for convicted offender samples in DNA Tracker will be updated to reflect dates of sample analysis (in progress, complete, failed as appropriate), date sample was outsourced for testing, and/or date profile was entered into CODIS. This is accomplished for individual samples under the 'Samples' tab for the Offender, by clicking on the appropriate sample, and choosing 'Add History Event'. Multiple samples may be updated using the 'Batch Samples' function at the top of the screen. The current disposition box must be checked as appropriate for the history(s) added.

CODIS DATABASE HIT VERIFICATION

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. This is accomplished by the electronic storage and maintenance of DNA profiles at the local, state and national levels. Hits are obtained when a candidate match(es) is identified through a database search at any level. Hit verification involves evaluating the candidate match to determine if it is a true match and verification of CODIS offender sample data where necessary and possible.

NDIS CODIS Hit Disposition Reporting & Confirm an Interstate Candidate Match - Operational Procedures

2.0 SCOPE:

To provide a method of sample verification to be performed prior to law enforcement agency notification of a database hit to ensure reporting of only true, confirmed matches.

3.0 EQUIPMENT/REAGENTS:

CODIS
NDIS Procedure Manual; CODIS Training Manual/documentation
DNA Tracker
AFIS
Offender Database Sample(s)
Equipment/Reagents for STR Analysis

4.0 PROCEDURE:

4.1 MATCH VERIFICATION (Forensic):

4.1.1 For 'hits'/matches involving an ISP Forensic Biology evidentiary sample (either case-to-case or case-to-offender) the primary responsibility for match verification follow-up and disposition lies with the Idaho CODIS Administrator.

4.1.2 The CODIS Administrator or designee (typically the case analyst) will first evaluate the 'Candidate Match' in Match Manager to determine if there is a basis for exclusion and, therefore, disposition as 'No Match'. A 'No Match' disposition doesn't require further verification or, where applicable, notification of the other laboratory involved with the match.

4.1.3 If evaluation in Match Manager demonstrates that the candidate match consists of potential high stringency (e.g., exclusions attributable to different typing systems, Promega v. Applied Biosystems), or possibly moderate stringency in the event of a forensic mixture or degraded sample, the disposition is changed from 'candidate Match' to 'Pending' until the verification process is complete. In general, for case-to-case matches, the verification will consist of communication between scientists regarding the data and case status, while case-to-offender matches typically necessitate sample verification at the 'offender lab'.

4.1.4 Once the status of the 'candidate match' has been resolved, the disposition is set accordingly (e.g., 'No Match', 'Offender Hit', 'Forensic Hit', 'Conviction Match', 'Investigative Information', etc.) and 'Investigations Aided' filled in as appropriate and as outlined in the NDIS 'CODIS Hit Disposition Reporting' procedure.

4.1.5 If verification results in a 'hit', a hit report is issued by the case analyst. A copy of the hit report, along with the CODIS match report, is filed in the CODIS file. The original hit report and a copy of the CODIS match report are placed in the associated case file. The appropriate law enforcement agency is notified of the 'hit'. If the law enforcement agency submits a sample from the identified offender, appropriate analysis and issuance of a supplemental report will be performed as in **4.2.5**.

4.2 MATCH VERIFICATION (Offender):

4.2.1 For 'hits'/matches involving an ISP Forensic Biology convicted offender sample the primary responsibility for match verification follow-up lies with the CODIS Administrator for the laboratory with the forensic (evidentiary) sample. However, the initial evaluation in Match Manager, (see **4.1.2-4**) and AFIS sample verification (see **4.2.2**) will be initiated as soon as feasible once a verification request has been received from the forensic laboratory.

4.2.2 Once a potential match has been confirmed and a verification request received, the associated offender sample folder will be retrieved from the secure file cabinet and taken to BCI for an AFIS search of the thumbprint to verify identification of the offender. All documentation will be filed in the CODIS file.

4.2.3 Following AFIS verification of the thumbprint, re-analysis of the offender sample will be performed as appropriate (i.e., if duplicate analysis has already been performed either as a QC function or as the result of a duplicate sample, analysis will not be repeated) prior to agency notification. In situations where a thumbprint was not received with the DNA sample, or is of insufficient quality for verification, a notation will be made and re-analysis for confirmation may proceed. The forensic laboratory (or law enforcement agency for Idaho cases) will be notified that the Offender could not be verified through thumbprint confirmation.

4.2.4 Following sample verification (AFIS and re-analysis as appropriate) the forensic case laboratory, in the case of an interstate hit, or submitting law enforcement agency will be notified of the confirmed hit. Laboratory notification may be made verbally and relevant documentation will be provided to the forensic case laboratory as requested. In Idaho, initial notification as well as the request for a new DNA sample from the identified offender, may be made verbally. However, written notification and a formal request for a new DNA sample, in the form of a hit report, will be sent to the appropriate law enforcement agency.

4.2.5 For intrastate offender hits (Idaho), where possible, a newly obtained DNA sample from the offender will be analyzed with all deliberate speed. The analysis of the forensic sample may also be repeated, though this is not required. A supplemental report will be issued delineating the match in the usual manner (i.e., same treatment as for matching suspect sample submitted with case evidence.

4.2.6 The sample history in DNA Tracker will be updated to reflect the date the hit was confirmed for the offender sample.

CODIS SAMPLE REMOVAL

1.0 BACKGROUND:

Participation in the National DNA database, in accordance with the DNA Analysis Backlog Elimination Act of 2000, necessitates provisions for DNA profile expungement in the event that a qualifying offender's conviction is overturned. Additionally, the Idaho DNA Database Act of 1996 addresses court-granted expungement requests (I.C. §19-5513). Removal of DNA profile data and/or destruction of biological samples obtained from Convicted Offenders may be necessary as a result of conviction reversal or sample collection/submission errors.

Expungement is defined as the removal of DNA profile data from local (LDIS), state (SDIS) and national (NDIS) databases in response to a court order overturning the offender's conviction of a qualifying offense. Expungement will include the removal of identifying information from other laboratory documentation and destruction of the biological sample from which the offender database DNA profile was generated.

Administrative removal is defined as the destruction of a DNA sample and removal of any records relating to that sample. Examples for which administrative removal may be warranted include, but are not limited to, the collection of a sample from a non-qualifying offender, or the notification by the collection agency that removal is warranted. Generally, the determination that a DNA sample does not qualify for inclusion in the database occurs prior to entry of the profile into DNA Tracker and subsequently CODIS (see BI-301); however, there may be instances when the collection agency provides notification of an error after the profile has been generated. In these circumstances, the profile will be removed from the local, state, and national databases as part of the administrative removal.

NDIS Expunge a DNA Profile - Operational Procedures

Idaho Statutes: Title 19, Criminal Procedure, Chapter 55 "The Idaho DNA Database Act of 1996"

2.0 SCOPE:

To provide a protocol for CODIS sample expungement and administrative removal that protects an individual's rights to privacy and maintains the integrity of the Idaho DNA database program. All procedures will be documented on the CODIS Sample Removal Checklist (Form 310-BI).

3.0 EQUIPMENT/REAGENTS:

CODIS Workstation
DNA Tracker Database

4.0 PROCEDURE:

4.1 EXPUNGEMENT

Prior to removal of any DNA profile data, source identification, or biological sample destruction, the CODIS Administrator or designee will verify: 1) the request for expungement is accompanied by a certified court order that meets the requirements outlined in I.C. §19-5513, 2) that the offender has no other qualifying offense(s) and 3) the identification of the original DNA database sample where possible.

4.1.1 A search of DNA Tracker is performed to establish whether or not the specified sample has been received in the laboratory and if so, whether or not a DNA profile has been generated and/or entered into CODIS.

4.1.2 If the sample has been received in the laboratory, a criminal history check in ILETS will be performed to ensure that the offense for which the expungement is requested is the only qualifying offense. If the Offender has a separate qualifying offense, the sample will not be expunged and a copy of the criminal history check will be retained for documentation.

4.1.3 The associated offender sample folder will be retrieved from the secure file cabinet and taken to BCI for an AFIS search of the thumbprint to verify identification of the offender. The absence of a thumbprint or a poor quality thumbprint does not preclude the sample expungement from proceeding.

4.1.4 The offender record will be removed from DNA Tracker and a sample removal report generated.

4.1.5 The DNA profile, if applicable, will be deleted from CODIS, followed by an upload to NDIS for removal at that level. A written notification of the expungement will be made to the NDIS Custodian and a request for verification of deletion at the national level.

4.1.6 The original DNA Collection Report received with the sample (or a copy of the sample folder when no report is received) will be marked expunged, along with the scientist's initials and date of expungement.

4.1.7 The DNA sample will be destroyed and witnessed by a second scientist.

4.1.8 Administrative paperwork documenting the event will be retained in the CODIS file. The paperwork may contain some administrative information about the sample/individual it was collected from and will include the following, as applicable: the court order/request for expungement, criminal history check and thumbprint verification, DNA Tracker sample removal report, CODIS deletion report(s), NDIS correspondence, CODIS sample removal checklist, and copies of any correspondence with the requesting party.

4.1.9 An expungement notification letter (or letter indicating why expungement did not occur), the DNA collection report or folder copy, and a copy of the CODIS sample removal checklist will be sent to the requesting party.

4.2 ADMINSTRATIVE REMOVAL

Prior to removal of any DNA profile data, source identification, or biological sample destruction, the CODIS Administrator or designee will verify: 1) the request for administrative removal is in writing from the collecting agency and includes a description of the error resulting in the removal request and 2) that the offender has no other qualifying offense(s).

Requests for administrative removal from a party other than the collecting agency will be referred to ISP legal staff for a determination of the appropriate action to be taken.

4.2.1 A search of DNA Tracker is performed to establish whether or not the specified sample has been received in the laboratory and if so, whether or not a DNA profile has been generated and/or entered into CODIS.

4.2.2 If the sample has been received in the laboratory, a criminal history check in ILETS will be performed to ensure that the offense for which the administrative removal is requested is the only qualifying offense. If the Offender has a separate qualifying offense, the sample will not be removed and a copy of the criminal history check will be retained for documentation.

4.2.3 The offender record will be removed from DNA Tracker and a sample removal report generated.

4.2.4 The DNA profile, if applicable, will be deleted from CODIS, followed by an upload to NDIS for removal at that level.

4.2.5 The original DNA Collection Report received with the sample (or a copy of the sample folder when no report is received) will be marked expunged, along with the scientist's initials and date of removal.

4.2.6 The DNA sample will be destroyed and witnessed by a second scientist.

4.2.7 Administrative paperwork documenting the event will be retained in the CODIS file. The paperwork may contain some administrative information about the sample/individual it was collected from and will include the following, as applicable: the request for administrative removal, criminal history check, DNA Tracker sample removal report, CODIS deletion report(s), CODIS sample removal checklist, and copies of any correspondence with the collection agency.

4.2.8 A sample removal notification letter (or letter indicating why removal did not occur), the DNA collection report or folder copy, and a copy of the CODIS sample removal checklist will be sent to the requesting party.

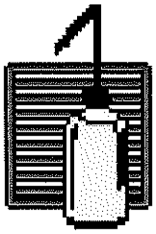
5.0 COMMENTS:

5.1 An Offender cannot be deleted from DNA Tracker if there are any samples and/or court orders associated with the offender.

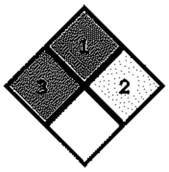
5.2 A sample cannot be expunged or deleted from DNA Tracker if there are qualifying offenses associated with the Offender.

5.3 The 'Delete Sample' function will be used to remove a sample from DNA Tracker for an Administrative Removal.

5.4 The 'Expunge Sample' function will be used to remove a sample from DNA Tracker for an Expungement.



Form 100-BI



PHENOLPHTHALEIN (KASTLE-MEYER) REAGENT

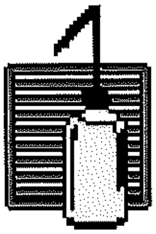
Phenolphthalein	2.0g
KOH	20.0g
Zinc (granular)	20.0g

Phenolphthalein, KOH, and 100mℓ of dH_2O are refluxed, in a fume hood, with Zinc until solution is colorless (producing phenolphthalin in ~4 hours). Store stock solution refrigerated in dark bottle to which ~5g mossy zinc has been added to keep the solution in its reduced form. Remove for working solution as needed.

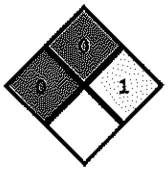
Working solution: Mix 2mℓ stock solution with 8mℓ Ethanol

Caution: Zinc is flammable. The unreacted portions and used filter paper are to be disposed of properly.

Date	Initials	Pheno Source/Lot#	KOH Source/Lot#	Zinc Source/Lot#	Ethanol Source/Lot#	Lab Lot# Reagent Name
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO
						PHENO



Form 102-BI



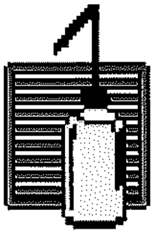
HYDROGEN PEROXIDE 3% (v/v)

Generally a commercial purchase, however, may be made from a 30% Solution (which is a commercial purchase) as follows:

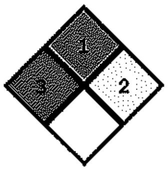
Hydrogen Peroxide (30%) 10mℓ/90mℓ nanopure dH_2O

Mix the H_2O_2 with 90mℓ of nanopure dH_2O and store at ~4°C.

Date	Initials	30% H_2O_2 Source/Lot#	Lab Lot# Reagent Name
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP



Form 103-BI

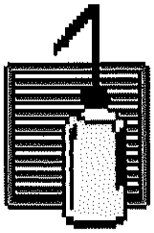


O-TOLIDINE REAGENT

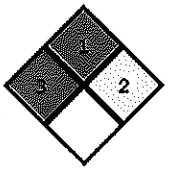
O-Tolidine	0.6g
Glacial Acetic Acid	100mℓ
Ethanol	100mℓ

Dissolve O-tolidine in Acetic Acid/Ethanol mixture consistent with ratios above. O-tolidine is light sensitive and should be stored in dark reagent bottle and kept refrigerated when not in use.

Date	Initials	O-tolidine Source/Lot	Acetic Acid Source/Lot#	Ethanol Source/Lot	Lab Lot# Reagent Name
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL



Form 104-BI



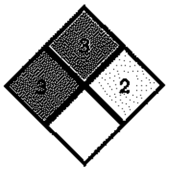
AMMONIUM HYDROXIDE (~3%)

Ammonium Hydroxide (Concentrated ~30%) 10mℓ/100mℓ

Add the NH_4OH to 90mℓ of nanopure dH_2O, mix well and store at RT.

Date	Initials	NH_4OH Source/Lot#	Lab Lot# Reagent Name
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH

Form 108-BI

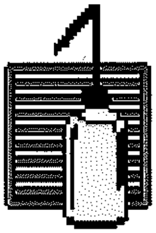


OUCHTERLONY DESTAIN

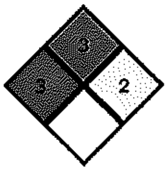
Methanol	45mℓ
Distilled water	45mℓ
Glacial Acetic Acid	10mℓ

Mix well and store refrigerated.

Date	Initials	MeOH Source/Lot#	Acetic Acid Source/Lot#	Lab Lot# Reagent Name
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD



Form 110-BI

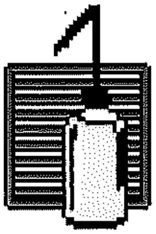


OUCHTERLONY STAIN

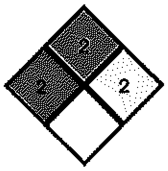
Ouchterlony Destain (Form 108-BI)	50mℓ
Coomassie Blue (Brilliant Blue R-250)	0.1g

Mix well (overnight), filter, and store at RT.

Date	Initials	Destain Lab Lot#	Stain Source/Lot#	Lab Lot# Reagent Name
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS



Form 114-BI

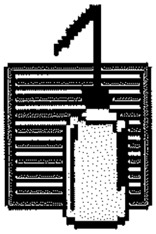


10X BRENTAMINE (SODIUM ACETATE) BUFFER

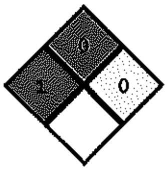
Sodium Acetate (Anhydrous)	1.2g
Acetic Acid(to adjust to pH 5)	≈400µℓ

Dissolve Sodium Acetate in 10mℓ of nanopure dH_2O. Add Acetic Acid to pH 5. Store refrigerated.

Date	Initials	Sodium Acetate Source/Lot#	Acetic Acid Source/Lot#	Lab Lot# Reagent Name
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT



Form 116-BI

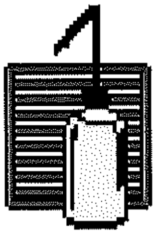


BRENTAMINE SOLUTION A

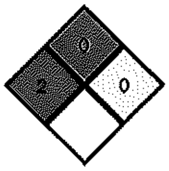
O-Dianisidine Tetrazotized (Fast Blue B Salt)	50 mg
10X buffer pH 5	5 mℓ

Dissolve Fast Blue B Salt in 5 mℓ of 10X Brentamine Buffer (Form 114-BI). Store refrigerated in a dark container.

Date	Initials	Fast Blue B Source/Lot#	10X Buffer Lab Lot#	Lab Lot# Reagent Name
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT



Form 118-BI

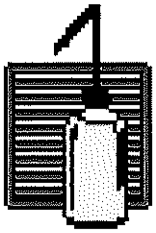


BRENTAMINE SOLUTION B

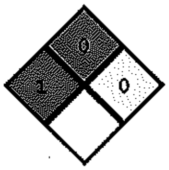
α-Naphthyl Phosphate (Disodium Salt) 50 mg

Dissolve in 5 mℓ of nanopure dH_2O. Store Refrigerated.

Date	Initials	α-naphthyl phosphate Source/Lot#	Lab Lot# Reagent Name
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT



Form 120-BI

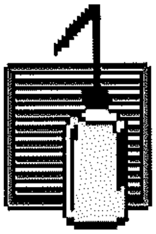


SALINE (0.85% NaCℓ)

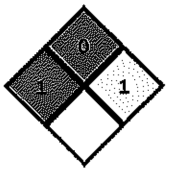
NaCℓ 4.25g/500mℓ

Dissolve the NaCℓ in 500 mℓ nanopure water. Sterilize by autoclaving. Store refrigerated.

Date	Initials	NaCl Source/Lot#	Lab Lot# Reagent Name
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ
			PNaCℓ



Form 124-BI



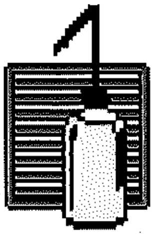
1X PHOSPHATE BUFFERED SALINE (PBS)

PBS 1 commercial pre-made packet

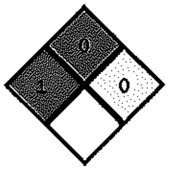
Dissolve one packet of powdered PBS in 1ℓ of nanopure dH_2O. Check that pH≅7.4, autoclave and store at RT.

If pre-made packets are not available, PBS may be prepared by dissolving 0.2g KCl, 8.0g NaCl, 0.2g KH_2PO_4, and 2.2g $Na_2HPO_4 \cdot 7H_2O$ (or 1.1g Na_2HPO_4 anhydrous) in 800mℓ nanopure dH_2O. Adjust pH to 7.4 if necessary. Q.S. to 1ℓ with nanopure dH_2O, autoclave and store at RT.

Date	Init.	1X PBS Src./Lot#	KCl Src./Lot#	NaCl Src./Lot#	KH_2PO_4 Src./Lot#	$Na_2HPO_4 \cdot 7H_2O$ Src./Lot#	Lab Lot# Reagent Name
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS



Form 126-BI



XMAS TREE STAIN SOLUTION A
(Kernechtrot Solution)

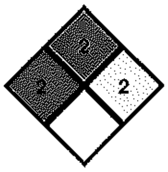
Aluminum Sulfate	5g
Nuclear Fast Red	0.1g

For 100mℓ, Dissolve the Aluminum Sulfate in 100mℓ **HOT** nanopure dH_2O. Immediately add the Nuclear Fast Red, mix, cool and filter (paper or ≥45µm). May be stored at RT.

May also be commercially purchased.

Date	Initials	Aluminum Sulfate Source/Lot#	Nuclear Fast Red Source/Lot#	Lab Lot# Reagent Name
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA

Form 128-BI



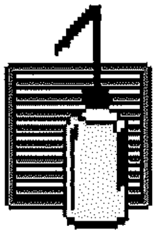
XMAS TREE STAIN SOLUTION B
(Picroindigocarmine Solution)

Saturated Picric Acid Solution	100mℓ
Indigo Carmine	0.33g

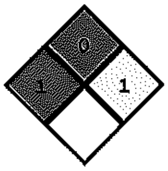
For 100mℓ, dissolve the Indigo Carmine in 100mℓ of the Picric Acid. Mix and filter (paper or ≥45µm). May be stored at RT.

May also be commercially purchased.

Date	Initials	Picric Acid Source/Lot#	Indigo Carmine Source/Lot#	Lab Lot# Reagent Name
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB



Form 132-BI

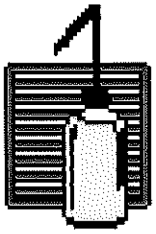


AMYLASE DIFFUSION/PHOSPHATE BUFFER (pH6.9)

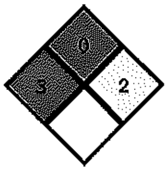
NaH_2PO_4, anhydrous	2.7g
Na_2HPO_4, anhydrous	3.9g
NaCℓ	0.2g

Mix the above with 500mℓ dH_2O, adjust pH to 6.9, and store at RT.

Date	Initials	NaH_2PO_4 Source/Lot#	Na_2HPO_4 Source/Lot#	NaCℓ Source/Lot#	Lab Lot# Reagent Name
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB



Form 134-BI

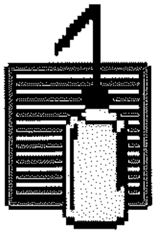


AMYLASE IODINE REAGENT

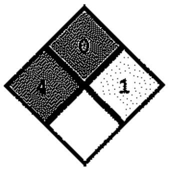
Potassium Iodide (KI)	1.65g
Iodine (I_2)	2.54g

Dissolve the above in 30mℓ nanopure dH_2O heated to ~65°C. Mix well, filter and store at 4°C in an amber bottle. Dilute 1:100 for Amylase Diffusion Test.

Date	Initials	KI Source/Lot#	I_2 Source/Lot#	Lab Lot# Reagent Name
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR



Form 138-BI

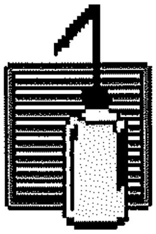


MERCURIC CHLORIDE 10% (w/v)

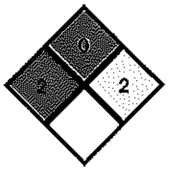
Mercuric Chloride	10g/100mℓ 95% EtOH

Dissolve the Mercuric Chloride in 100mℓ of 95% Ethanol, mix well and store at RT.

Date	Initials	EtOH Source/Lot#	Mercuric Chloride Source/Lot#	Lab Lot# Reagent Name
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC



Form 140-BI

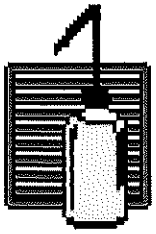


ZINC CHLORIDE 10% (w/v)

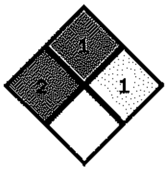
Zinc Chloride 10g/100mℓ 95% EtOH

Dissolve the Zinc Chloride in 100mℓ of 95% Ethanol, mix well and store at RT.

Date	Initials	EtOH Lot# Source/Lot#	Zinc Chloride Source/Lot#	Lab Lot# Reagent Name
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC



Form 201-BI

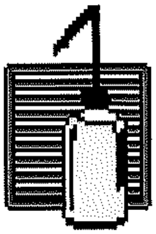


1M TRIS-HCℓ Buffer pH7.5

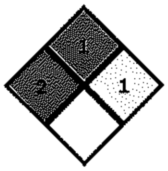
Tris Base(tris[Hydroxymethyl]amino methane)	121.1 g

Dissolve Tris in ~800 mℓ nanopure dH_2O. Adjust to pH7.5 at RT by adding concentrated HCℓ (approximately 65mℓ). Q.S. to 1ℓ with nanopure dH_2O, autoclave and store at RT.

Date	Initials	Tris Base Source/Lot #	HCℓ Source/Lot#	Lab Lot# Reagent Name
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5



Form 203-BI

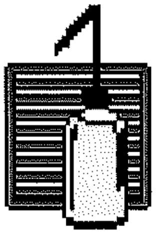


1M TRIS-HCℓ Buffer pH8

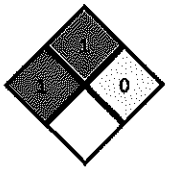
Tris Base(tris[Hydroxymethyl]amino methane) 121.1 g

Dissolve Tris in ~800 mℓ nanopure dH$_2$O. Adjust to pH8 at RT by adding concentrated HCℓ (approximately 45mℓ). Q.S. to 1ℓ with nanopure dH$_2$O, autoclave and store at RT.

Date	Initials	Tris Base Source/Lot#	HCℓ Source/Lot#	Lab Lot# Reagent Name
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8



Form 205-BI



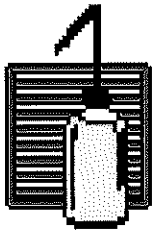
ETHYLENEDIAMINE TETRAACETIC ACID (EDTA) 0.5M

$Na_2EDTA \cdot 2H_2O$ 186.1g/ℓ

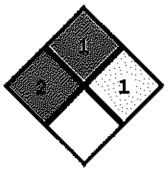
Slowly add EDTA to 800mℓ nanopure H_2O while stirring vigorously. Add ~20g of NaOH pellets to bring the pH to near 8.0. When fully dissolved adjust pH to 8.0 and bring final volume to 1ℓ. Autoclave and store at RT.

Note: EDTA will not go into solution without the pH adjustment.

Date	Initials	EDTA Source/Lot#	NaOH Source/Lot#	Lab Lot# Reagent Name
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA



Form 207-BI



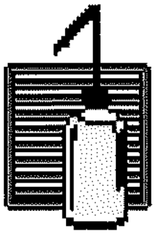
STAIN EXTRACTION BUFFER pH8
(10mM EDTA, 10mM Tris-HCℓ, 50mM NaCl, 2% SDS)

1M Tris-HCℓ, pH7.5	5mℓ
0.5M EDTA	10mℓ
5.0M NaCℓ	5mℓ
10% SDS	100mℓ

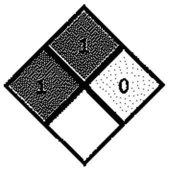
Mix the Tris-HCℓ, EDTA, NaCℓ and SDS with ~380mℓ nanopure dH_2O. Store at RT.

Note: Reagent contains SDS, do not autoclave.

Date	Initials	Tris-HCl Source/Lot#	EDTA Source/Lot#	NaCl Source/Lot#	SDS Source/Lot#	Lab Lot# Reagent Name
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB



Form 211-BI



PROTEINASE K (20mg/mℓ)

Commercial Purchase of 20mg/mℓ Solution dispensed and stored as indicated below.

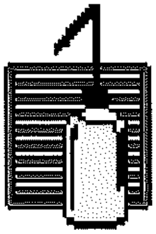
or

Proteinase K 0.2g

Dissolve the ProK in 10mℓ sterile nanopure dH_2O.

Dispense ~500µℓ (commercial purchase or in-house prep.) each into sterile microfuge tubes and store at ≤20°C.

Date	Initials	ProK Source/Lot#	Lab Lot# Reagent Name
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK



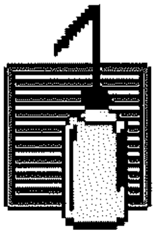
Form 222-BI

1M Sodium Acetate, pH5.2

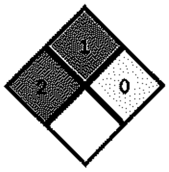
$CH_3COONa \cdot 3H_2O$ 13.6g

Dissolve the $CH_3COONa \cdot 3H_2O$ in 80mℓ nanopure dH_2O. Adjust to pH5.2 by adding glacial acetic acid (approximately 2 mℓ). Q.S. to 100mℓ with nanopure dH_2O, autoclave and store at RT.

Date	Initials	$CH_3COONa \cdot 3H_2O$ Source/Lot #	Acetic Acid Source/Lot #	Lab Lot# Reagent Name
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA



Form 223-BI



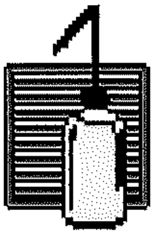
DTT SOLUTION

Dithiothreitol (DTT) 0.77g

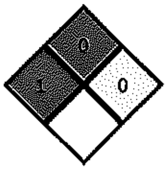
Dissolve the DTT in 5mℓ nanopure dH_2O. Add 50μℓ 1M Sodium Acetate, pH5.2. Dispense ~500μℓ each into sterile microcentrifuge tubes and store at ≅20°C.

Note: Do not autoclave.

Date	Initials	DTT Source/Lot #	Sodium Acetate Source/Lot #	Lab Lot# Reagent Name
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT
				DTT



Form 225-BI



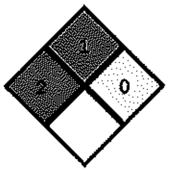
CHELEX REAGENT 5%

Chelex 0.5g/10mℓ

Dissolve the Chelex in 10mℓ sterile nanopure dH_2O. This solution should be freshly prepared prior to use and the remaining solution discarded after ≤3 days in refrigeator.

Date	Initials	Chelex Source/Lot#	Lab Lot# Reagent Name
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE

Form 229-BI

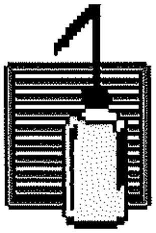


PCR-TE (TE^{-4}) BUFFER
(10mM Tris-HCl, 0.1mM EDTA)

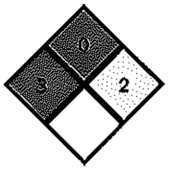
1M Tris-HCl, pH8	10mℓ
0.5M EDTA, pH8	0.2mℓ

Mix Tris-HCl and EDTA with 990mℓ nanopure dH_2O. Autoclave and store at RT.

Date	Initials	1M Tris-HCl Source/Lot#	0.5M EDTA Source/Lot#	Lab Lot# Reagent Name
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE



Form 231-BI



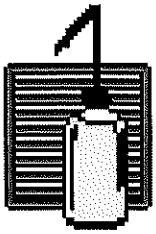
NaOH 5N

NaOH	50g

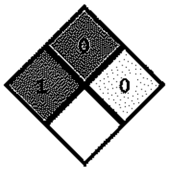
Slowly dissolve the Sodium Hydroxide in 250mℓ sterile nanopure dH_2O. Allow to cool and store at RT.

Caution: NaOH is highly caustic. This reaction generates heat.

Date	Initials	NaOH Pellets Source/Lot#	Lab Lot# Reagent Name
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH
			NaOH



Form 233-BI



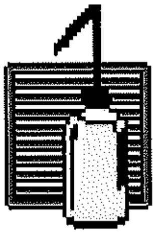
SODIUM CHLORIDE (NaCℓ) 5M

NaCℓ 146.1g/500mℓ

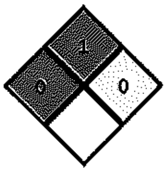
Dissolve the NaCℓ in 500 mℓ nanopure water. Sterilize by autoclaving.

May also be purchased as 5M solution.

Date	Initials	NaCl Source/Lot#	Lab Lot# Reagent Name
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ
			NaCℓ



Form 249-BI



BOVINE SERUM ALBUMIN (BSA) 4%

BSA	0.4 g
PCR-TE	10 mℓ

Dissolve the BSA in PCR-TE. Filter-sterilize and dispense ~500μℓ each into 1.5mℓ microfuge tubes. Store at ~-20°C.

Date	Initials	BSA Source/Lot#	PCR-TE Source/Lot#	Lab Lot# Reagent Name
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA

Form 101-BI

Scientist: ________

Case Number: ________________

Date: __________

BIOLOGY SCREENING SUMMARY

SAMPLE ID	BLOOD		SEMEN			SALIVA	URINE	FECES
	Chemical	Hematrace	P30	AP	MICROSCOPIC EXAM			

Form 200-BI

DNA EXTRACTION WORKSHEET

Scientist ____________________ **Case#** ____________________

Blood/Saliva Extraction

Date __________

Items

Lot #

1a. 500µℓ SEB SEB ____________________
1b. 15µℓ Pro K ProK ____________________
2. 200µℓ Chelex Che ____________________
3a. 150µℓ FTA FTA ____________________
3b. 150µℓ TE TE ____________________

EZ1 Extraction

Date __________

1a. ___µℓ SEB SEB ____________________
1b. 10µℓ Pro K ProK ____________________
1C. EZ1 Kit EZ1 ____________________

Hair Extraction

Date __________

1a. 500µℓ SEB SEB ____________________
1b. 20µℓ DTT DTT ____________________
1c. 15µℓ Pro K ProK ____________________

Bone/Teeth Extraction

Date __________

1a. 500µℓ SEB SEB ____________________
1b. 15µℓ Pro K ProK ____________________

Centricon Concentration

Date __________

1a. 500µℓ PCIAA PCIAA ____________________
1b. TE TE ____________________

Form 202-BI

DIFFERENTIAL DNA EXTRACTION WORKSHEET

Scientist ____________________ **Case#** ____________________

Differential Extraction (EC)

Date __________

Lot #

Items

1a. 150µℓ PBS PBS ____________________

1b. 500µℓ SEB SEB ____________________

1c. 15µℓ Pro K ProK ____________________

Differential Extraction (SP)

Date __________

1a. 1000µℓ PBS PBS ____________________

1b. 1000µℓ dH_2O

1c. 500µℓ SEB SEB ____________________

1d. 20µℓ DTT DTT ____________________

1e. 15µℓ Pro K ProK ____________________

Centricon Concentration

Date __________

1a. 500µℓ PCIAA PCIAA ____________________

1b. TE TE ____________________

DNA Quantitation

7000 Load Sheet

Form 206-BI

Case Number: ____________________

Analyst: __________

Plate Name: ____________________

Date: __________

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD. 1	STD. 1										
B	STD. 2	STD. 2										
C	STD. 3	STD. 3										
D	STD. 4	STD. 4										
E	STD. 5	STD. 5										
F	STD. 6	STD. 6										
G	STD. 7	STD. 7										
H	STD. 8	STD. 8										

Quantifiler Kit

lot #: ____________________

expiry date: ____________________

total samples: __________

Std. Prep. Date: ____________________

TE lot#: ____________________

Master Mix made for: __________

reaction mix	**0** ul
primer mix	**0** ul

DNA Quantitation

7000 Results Sheet

Form 209-BI

Case Number: ________________ Analyst: __________

Plate Name: ________________ Date: __________

Well	Sample Name	IPC C_T	Quantity ng/ul	ul Sample for Dilution	ul TE to be added	ng/ul Final	ul to be Amplified
A3	0	0	0	5	0.0	0.1	10.0
B3	0	0	0	5	0.0	0.1	10.0
C3	0	0	0	5	0.0	0.1	10.0
D3	0	0	0	5	0.0	0.1	10.0
E3	0	0	0	5	0.0	0.1	10.0
F3	0	0	0	5	0.0	0.1	10.0
G3	0	0	0	5	0.0	0.1	10.0
H3	0	0	0	5	0.0	0.1	10.0
A4	0	0	0	5	0.0	0.1	10.0
B4	0	0	0	5	0.0	0.1	10.0
C4	0	0	0	5	0.0	0.1	10.0
D4	0	0	0	5	0.0	0.1	10.0
E4	0	0	0	5	0.0	0.1	10.0
F4	0	0	0	5	0.0	0.1	10.0
G4	0	0	0	5	0.0	0.1	10.0
H4	0	0	0	5	0.0	0.1	10.0
A5	0	0	0	5	0.0	0.1	10.0
B5	0	0	0	5	0.0	0.1	10.0
C5	0	0	0	5	0.0	0.1	10.0
D5	0	0	0	5	0.0	0.1	10.0
E5	0	0	0	5	0.0	0.1	10.0
F5	0	0	0	5	0.0	0.1	10.0
G5	0	0	0	5	0.0	0.1	10.0
H5	0	0	0	5	0.0	0.1	10.0
A6	0	0	0	5	0.0	0.1	10.0
B6	0	0	0	5	0.0	0.1	10.0
C6	0	0	0	5	0.0	0.1	10.0
D6	0	0	0	5	0.0	0.1	10.0
E6	0	0	0	5	0.0	0.1	10.0
F6	0	0	0	5	0.0	0.1	10.0
G6	0	0	0	5	0.0	0.1	10.0
H6	0	0	0	5	0.0	0.1	10.0
A7	0	0	0	5	0.0	0.1	10.0
B7	0	0	0	5	0.0	0.1	10.0
C7	0	0	0	5	0.0	0.1	10.0
D7	0	0	0	5	0.0	0.1	10.0
E7	0	0	0	5	0.0	0.1	10.0
F7	0	0	0	5	0.0	0.1	10.0
G7	0	0	0	5	0.0	0.1	10.0
H7	0	0	0	5	0.0	0.1	10.0
A8	0	0	0	5	0.0	0.1	10.0
B8	0	0	0	5	0.0	0.1	10.0
C8	0	0	0	5	0.0	0.1	10.0
D8	0	0	0	5	0.0	0.1	10.0
E8	0	0	0	5	0.0	0.1	10.0
F8	0	0	0	5	0.0	0.1	10.0
G8	0	0	0	5	0.0	0.1	10.0

Well	Sample Name	IPC C_T	Quantity ng/ul	ul Sample for Dilution	ul TE to be added	ng/ul Final	ul to be Amplified
H8	0	0	0	5	0.0	0.1	10.0
A9	0	0	0	5	0.0	0.1	10.0
B9	0	0	0	5	0.0	0.1	10.0
C9	0	0	0	5	0.0	0.1	10.0
D9	0	0	0	5	0.0	0.1	10.0
E9	0	0	0	5	0.0	0.1	10.0
F9	0	0	0	5	0.0	0.1	10.0
G9	0	0	0	5	0.0	0.1	10.0
H9	0	0	0	5	0.0	0.1	10.0
A10	0	0	0	5	0.0	0.1	10.0
B10	0	0	0	5	0.0	0.1	10.0
C10	0	0	0	5	0.0	0.1	10.0
D10	0	0	0	5	0.0	0.1	10.0
E10	0	0	0	5	0.0	0.1	10.0
F10	0	0	0	5	0.0	0.1	10.0
G10	0	0	0	5	0.0	0.1	10.0
H10	0	0	0	5	0.0	0.1	10.0
A11	0	0	0	5	0.0	0.1	10.0
B11	0	0	0	5	0.0	0.1	10.0
C11	0	0	0	5	0.0	0.1	10.0
D11	0	0	0	5	0.0	0.1	10.0
E11	0	0	0	5	0.0	0.1	10.0
F11	0	0	0	5	0.0	0.1	10.0
G11	0	0	0	5	0.0	0.1	10.0
H11	0	0	0	5	0.0	0.1	10.0
A12	0	0	0	5	0.0	0.1	10.0
B12	0	0	0	5	0.0	0.1	10.0
C12	0	0	0	5	0.0	0.1	10.0
D12	0	0	0	5	0.0	0.1	10.0
E12	0	0	0	5	0.0	0.1	10.0
F12	0	0	0	5	0.0	0.1	10.0
G12	0	0	0	5	0.0	0.1	10.0
H12	0	0	0	5	0.0	0.1	10.0

Form 210-BI

STR AMPLIFICATION SET-UP

Date:__________ Scientist:__________ STR Kit Type:__________

STR Kit Lot:__________ Taq Lot:__________

Reagent	µℓ/sample	X	Master Mix #Samples	=	µℓ in Master
Rxn Buffer	µℓ				
Primers	µℓ				
H_2O	µℓ				
Taq Gold	µℓ				
Master Mix/Sample	µℓ				
DNA Template	µℓ				
Total Rxn Volume	µℓ				

PCR TE Lot#__________

Case(s)

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Front

Form 212-BI

STR BLIND CONTROL GENOTYPE CHECK

Blind Control Number:______________

Date:____________

LOCUS	ALLELES	LOCUS	ALLELES
D3S1358	√	THO1	√
D21S11	√	D18S51	√
Penta E	√	D5S818	√
D13S317	√	D7S820	√
D16S539	√	CSF1PO	√
Penta D	√	Amelogenin	√
vWA	√	D8S1179	√
TPOX	√	FGA	√

Correct Genotype ☒

Reviewer's Initials ________

Comments:

Form 214-BI

STR Technical Review Checklist

ase Number:____________ Reviewer's Initials:________ Date:__________

Is the following paperwork included in the case file?

☐ Case Notes
☐ Extraction Worksheet
☐ 7000 Worksheets/Standard Curve
☐ DNA Concentration Worksheet
☐ Amplification Worksheet
☐ 3130 Load Sheet
☐ GeneMapper ID Electropherogram Plots
☐ Allelic Table
☐ CODIS Entry Form

Data Review:

☐ Correct assignment of size standard peaks.

☐ Positive Control appears as expected in GeneMapper ID.

☐ No allelic peaks or unacceptable artifacts found in Negative Controls.

☐ No unacceptable matrix problems (e.g., excessive pull-up or baseline problems).

☐ Correct genotypic assignment of ladder alleles.

☐ Sample plots examined for proper genotype and off-ladder assignments.

☐ Verify Genotypic result of positive control(s), negative control(s), and sample(s).

☐ GeneMapper ID plot results and table results are in agreement.

☐ Statistical Analysis appropriate and correct alleles used.

☐ Conclusion(s) are supported by results/data.

☐ Report addresses all items or probative fraction(s) tested.

☐ Unidentified profile(s) compared to batch and staff profiles.

☐ Profiles eligible for CODIS entry correctly identified and correct genotypes and specimen categories assigned.

Comments:

3130 Load Sheet

Form 216-BI

Case Number: ____________________

Plate Name: ____________________

Analyst: __________

Date: __________

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

total samples: __________

Master Mix made for: __________

3130 POP4

Lot# ____________________

Expiration Date ____________________

HiDi Formamide Lot# ____________________

Buffer Lot# ____________________

HiDi Formamide	**0** ul
Internal Lane Standard	**0** ul

Revision 7
2/4/2008
Issuing Authority:Quality Manager

Form 218-BI

CODIS Entry Form

Case Number:____________ Scientist:________ Date:__________

Profile(s) eligible for entry into CODIS? ☐ Yes ☐ No
(If yes, fill out the remainder of the form)

	Sample ID	**Specimen Category**	**Source ID'd (yes/no)**
1)			
2)			
3)			
4)			
5)			
6)			
7)			
8)			
9)			
10)			

☐ Forensic profile(s) associated with a crime
☐ Forensic profile(s) believed to be attributable to the putative perpetrator
☐ Forensic profile(s) does not match Victim and/or Elimination Samples
☐ Forensic Mixture(s) meets 4 X 4 rule requirements

Indexes and Associated Categories Acceptable for Entry

Index	**Specimen Category**
Forensic	Forensic Unknown, Forensic Mixture
Unidentified Human (Remains)	Unidentified Human Remains, Unidentified Person
Relatives of Missing Person	Biological Mother/Father/Child/Sibling, Maternal Relative, Paternal Relative
Missing Person	Deduced Victim Known, Missing Person

Comments:

eviewer's Initials:_______ Date:_______

Form 306-BI

STR CODIS Review Checklist

CODIS Run:____________ Reviewer's Initials:________ Date:__________

Is the following paperwork included in the CODIS Data file?

☐ Extraction Worksheet
☐ Amplification Worksheet
☐ 3130 Load Sheet
☐ GeneMapper ID Electropherogram Plots
☐ Allelic Table

Data Review:

☐ Correct assignment of size standard peaks.

☐ Positive Control appears as expected in GeneMapper ID.

☐ No allelic peaks or unacceptable artifacts found in Negative Controls.

☐ No unacceptable matrix problems (e.g., excessive pull-up or baseline problems).

☐ Correct genotypic assignment of ladder alleles.

☐ Sample plots examined for proper genotype and off-ladder assignments.

☐ Verify Genotypic result of positive control(s), negative control(s), and sample(s).

☐ GeneMapper ID plot results and table results are in agreement.

☐ Data certified for upload to CODIS.

Comments:

Form 310-BI

CODIS SAMPLE REMOVAL CHECKLIST

Requesting Party: ______________________________

Offender Name or Number: ______________________________

☐ Written request for expungement present with certified court order meeting requirements of I.C. §19-5513.

or

Written request for administrative removal from collection agency present with description of error made.

☐ Offender's sample received in the laboratory.

☐ Offender has no other qualifying offenses.

☐ Thumbprint on sample verified (expungement requests).

☐ All electronic data/information relating to the individual deleted from DNA Tracker.
Date: ______________ Initials: __________

☐ DNA profile deleted from CODIS and NDIS upload performed to delete from the national level.
Date: ______________ Initials: __________

☐ The DNA sample being expunged/removed destroyed in the presence of a second scientist.
Date: ______________ Initials: __________ Witness Initials: __________

☐ All administrative paperwork documenting the removal event retained in the CODIS file.

☐ Original collection report (or copy of sample folder) marked expunged to be returned to requesting party with letter and copy of checklist.

These actions comply with the ISP Biology Analytical Methods Manual (procedure BI-310) covering CODIS Sample Removal and satisfy all federal requirements.

Form 400-QC

Forensic Biology Chemical Inventory

Chemical	Source	Grade	Lot Number	Order Date	Exp. Date	Date Rcvd	Qty Rcvd	Qty In Stock

Last Audited ____________

Chemical	Source	Grade	Lot Number	Order Date	Exp. Date	Date Rcvd	Qty Rcvd	Qty In Stock

Form 401-QC

Forensic Biology Critical Equipment Inventory

Manufacturer/Model/Instrument	Software/version	Serial #	Property #	Location	Validation Date

Note: completed validation indicates the instrument complies with all specifications and/or methods

Form 402-QC

FORENSIC BIOLOGY EQUIPMENT MAINTENANCE/REPAIR RECORD

FILL IN ALL AVAILABLE INFORMATION.

Equipment Name/Description: ____________________

Serial Number: (if multiple, e.g., list all or reference)

Scheduled Maintenance/Repair (circle one)

If scheduled maintenance, brief description of maintenance type (i.e. annual PM) or reference to vendor report if applicable and date next maintenance due:

If repair, brief description of identified problem:

Vendor/Individual Performing Action: ____________________

Date Action Performed: ____________________

Result: Completed / taken out of service / sent out for calibration|repair / other (circle one)

Comments:

Form 403-QC

FORENSIC BIOLOGY pH CALIBRATION RECORD

(Oakton pH meter, serial #135212)

DATE	INITIALS	STANDARD BUFFER pH 4.01 Reading/lot #	STANDARD BUFFER pH 7.00 Reading/lot #	STANDARD BUFFER pH 10.01 Reading/lot #

A 3-point calibration of the pH meter will be performed at the time of use (*See the Oakton Operating Manual for calibration and pH measurement instructions*). The analyst will record the date of calibration, their initials, the measured pH value and lot # for each buffer. The measured reading must fall within ±0.50 pH for the calibration to be confirmed by the meter.

Form 404A-QC

FORENSIC BIOLOGY WEEKLY QC

DATE/INITIALS												
	°C	Min	Max	°C	Min	Max	°C	Min	Max	°C	Min	Max
COMBO F/F A												
FRIDGE A												
FREEZER A												
FREEZER B												
FREEZER C												
COMBO F/F B												
COMBO F/F C												

Observed temperatures should fall between ±5°C of the target temperature (4°C for refrigerators and –20°C for freezers). The temperature control should be adjusted to correct for minor variations; however, If the temperature is not corrected or if it falls significantly outside the target range, it should be taken out of service and maintenance/repair performed as needed. Note: frost-free freezers will have a greater temperature range (±10°C) due to the heating and cooling cycles. Combination fridge/freezers with a single temperature control may not be able to maintain both units within the given range. In this case, temperature sensitive reagents should be stored in appropriate containers (such as cryo-boxes) to maintain the desired state.

DATE/INITIALS				
NANOPURE SYSTEM				
°C OVEN **set/observed**				
°C HEATING BLOCK A **set/observed**				
°C HEATING BLOCK B **set/observed**				
°C HEATING BLOCK C (prod. rm.) **set/observed**				
°C HEATING BLOCK D **set/observed**				

Observed temperatures should fall between ±2°C of the temperature set point. The temperature control should be adjusted to correct for minor variations; however, If the temperature is not corrected or if it falls significantly outside the target range, it should be taken out of service and maintenance/repair performed as needed.

The observed water purity for the Nanopure system should be a minimum of 18.0 mega-ohms. If the purity falls below this point, the cartridges should be changed and the system sanitized as necessary.

Form 404B-QC

MERIDIAN EVIDENCE VAULT WEEKLY QC

DATE/INITIALS												
	˚C	Min	Max	˚C	Min	Max	˚C	Min	Max	˚C	Min	Max
FRIDGE 1												
FRIDGE 2												
FREEZER 1												
FREEZER 2												
FREEZER 3												
DNA FREEZER 1												
DNA FREEZER 2												
DNA FREEZER 3												

Observed temperatures for refrigerators should fall between ±5°C of the 4°C target temperature. Freezers should fall between ±10°C of the target –20°C. The temperature control should be adjusted to correct for minor variations; however, If the temperature is not corrected or if it falls significantly outside the target range, it should be taken out of service and maintenance/repair performed as needed. Note: frost-free freezers will have a greater temperature range (±15°C) due to the heating and cooling cycles.

FORENSIC BIOLOGY MONTHLY QC

Form 406A-QC

DATE/INITIALS												
AUTOCLAVE												
CLEAN												
STERILIZATION (+)												
(-)												
LABORATORY AND OTHER EQUIPMENT												
BIOROBOT EZ1s GREASE D-RINGS												
CLEAN CENTRIFUGES												
CLEAN PIPETS												
LAB CLEANED												

*Personnel should initial the duties they perform and date separately, if necessary.

Autoclave sterilization is checked by the observation of microbial growth in the (+) control (non-sterilized) and a lack of growth in the (-) control (sterilized) samples. See the BTSure product insert for test instructions and growth indicators. If sterilization is not achieved, the autoclave should be serviced.

FORENSIC BIOLOGY MONTHLY QC

Form 406B-QC

DATE/INITIALS												
ABI 7000 Instrument Maintenance												
Background Assay												
Block Contamination Check												
Lamp Function Test/ Illumination												
7000 & 310 Computer Maintenance												
Disk Cleanup												
Defragment Hard Drive												
3130 Maintenance												
Water Seal Trap Flush												
Water Wash Wizard												
Defragment Hard Drive (C & E)												

*Personnel should initial the duties they perform and date separately, if necessary.

Note: See the ABI 7000 User Guide and/or the April 2007 User Bulletin for Instrument Maintenance procedures and pass/fail criteria.

If outliers are observed during the Background Assay (Intensity Value ≥1200), or fluorescence (red) observed during the block check, the specific well should be identified and cleaned. Recheck the block after cleaning. If the lamp fails the function test and/or illumination check, it should be replaced, followed by calibration of ROI, pure dye, and instrument verification.

The 7000 and 310 Disk Cleanup is performed by selecting Start Menu > Programs > Accessories > System Tools > Disk Cleanup.

7000 and 310 Defragmentation is performed by selecting Start Menu > Programs > Accessories > System Tools > Disk Defragmenter.

3130 Defragmentation is performed by right-clicking on 'My Computer' and selecting 'Manage'. In the tree tab choose Computer Management (local)>Disk Fragmenter>Drive name>Defragment.

Form 408A-QC

FORENSIC BIOLOGY QUARTERLY QC

VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 9700

DATE: ________________
TESTED BY: ________________
THERMAL CYCLER ID: ________________
PROBE SERIAL # 6000029
THERMOMETER SERIAL # 00D400195

See User's Manual for test procedures.

Temperature Non-Uniformity

SETPOINT VALUE	94 *C	37 *C
A1		
A12		
C4		
C9		
F4		
F9		
H1		
H12		
TNU AT 94°C: _____ PASS FAIL		
TNU AT 37°C: _____ PASS FAIL		

Calibration Verification

SETPOINT VALUE			PASS
A6	85°C	45°C	
			FAIL

PLACE DOCUMENT IN QC BINDER

Form 408B-QC

FORENSIC BIOLOGY QUARTERLY QC

SCIENTIST:___________ QC DATE:_____________

IV. Chemical Safety Shower Check ________

V. Chemical Safety Eye Wash Station Check ________

Form 410-QC

QC ABACARD® HEMATRACE® KIT

HEMATRACE® KIT LOT:___________ **DATE RECEIVED:**___________

SCIENTIST:__________ **QC DATE:**____________

Perform test as usual with one 2mm^2 cutting and one 2mm thread from known bloodstain. Record results (include time it took for positive rxn to be visible). If available, attach photo documentation and place in Forensic Biology QC binder.

SAMPLE	RXN	TIME (min. sec.)
2mm^2 cutting		
2mm thread		
Neg		

The 2mm^2 cutting sample must have a positive reaction within 10 minutes for passing. The 2mm thread should ideally be positive within 10 minutes but is used primarily as a sensitivity indicator of the given test lot. The kit may still be deemed as passing without a positive result for the thread.

QA/QC PASSED: YES ☐ **NO** ☐

Comments:

Form 412-QC

QC OneStep ABACARD® p30 KIT

ABACARD® p30 KIT LOT:_______________ **DATE RECEIVED:**___________

SCIENTIST:__________ **QC DATE:**____________

Perform test as usual with a known semen extract, as well as ~10ng/mℓ (10μℓ of a 1:500 dilution) and ~50ng/mℓ (10μℓ of a 1:100 dilution) of Seri Semen Standard. Record results (include time it took for positive rxn to be visible). If available, attach photo documentation and place in Forensic Biology QC binder.

SAMPLE	RXN	TIME (min. sec.)
Semen Extract		
10ng/mℓ		
50ng/mℓ		
Neg		
*250ng/mℓ or 1:10		

The semen extract must have a positive rxn within 10 minutes for passing. The Seri standards are used to estimate the range of sensitivity of the kit lot.

For the semen standard dilutions, if a positive rxn is not obtained at 10 minutes, continue to monitor and record result at the end of 15 minutes. In addition, *run a 250ng/mℓ (50μℓ of the 1:100 dilution to 150μℓ of extraction buffer) **or** a 1:10 dilution of the semen stain extract to ensure the kit is operating within reasonable limits for forensic identification. In addition to the neat semen extract, this control sample (250ng/mℓ or 1:10 extract) must result in a positive rxn within 10 minutes.

QA/QC PASSED: YES ☐ **NO** ☐

Comments:

Form 419-QC

QC QUANTIFILER HUMAN KITS

KIT LOT #: ____________________ **DATE RECEIVED:** ________________

EXPIRATION DATE: ______________

SCIENTIST: ________________ **QA/QC DATE:** ______________

KIT COMPONENT	LOT NUMBER
PRIMER MIX	
REACTION MIX	
DNA STANDARD	

To check the new kit lot, perform quantification as usual. For samples, run standards from the new kit to be QC'd and equivalent dilutions of the NIST SRM 2372 Quant Standard, as well as 0.5ng and 10ng of 9947A DNA. Analyze using the SRM as standard and the new kit as unknown. Using an average of the results for the new kit standards, calculate the new volume of TE to be added in the preparation of standard 1, per the equation $C_1V_1=C_2V_2$ (where C=average for std 1, and V=total volume). Record the slope obtained for the standard curve.

As a check of the calculation and resulting TE volume, use the new kit, with corresponding new dilution to perform a 9947A DNA quantification, according to standard procedure. Use 0.5ng and 10ng and compare the results to those obtained from above. A pass will be achieved if the slopes for both standard curves are comparable.

SRM 2372 component used: ____________
Standard curve slope: ____________
Volume TE to be used for Standard 1: ____________

QA/QC PASSED: YES ☐ **NO** ☐

Comments:

Attach the 7000 Load Sheets, Standard Curves, and Results Sheets. Record the calculations in the documentation. Mark the new kit with TE volume for Standard 1 preparation.

Form 420-QC

QC STR KITS

STR KIT: ____________ **DATE RECEIVED:** ____________

KIT MANUFACTURER: ____________ **KIT LOT #:** ____________

LAB LOT#: ____________ **SCIENTIST:** ____________ **QA/QC DATE:** ____________

KIT COMPONENT	LOT NUMBER
PRIMER MIX	
REACTION MIX	
CONTROL DNA	
TAQ GOLD*	
ALLELIC LADDER	

Perform extraction of one Blind Control and amplify as usual with reagent blank and controls. GeneMapper® ID data will be analyzed as usual and the quality of results reflected in the comments section as appropriate and necessary. A pass will be achieved by obtaining the expected results for each of the samples run and data of acceptable quality (e.g. sufficient RFUs).

* Taq Gold is purchased separately, but typically at the same time as a new STR kit. If Taq Gold is received separate from an STR kit, the QC of the Taq will be noted on this form (corresponding to the appropriate STR kit lot#) under comments.

Run Date: ____________ **Run Folder:** ____________

QA/QC PASSED: **YES** ☐ **NO** ☐

Comments:

Attach the appropriate extraction/amplification/BC forms used and the GeneMapper ID Electropherograms; place in Forensic Biology QC Binder.

Form 422A-QC

310 INJECTION LOG

DATE	ANALYST	FORMAMIDE (LOT#)	H_2O CHANGED √	BUFFER CHANGED √ (LOT#)	310 POP-4 CHANGED √ (LOT#/EXP)	#INJECTIONS RUN/TOTAL	CAPILLARY CHANGED √ (LOT#)	CASES RUN

Form 422B-QC

3130 INJECTION LOG

DATE	ANALYST	H_2O WASH √	H_2O TRAP FLUSH √	H_2O CHANGED √	BUFFER CHANGED √ (LOT#)	3130 POP-4 CHANGED √ (LOT#/EXP)	CAPILLARY ARRAY CHANGED √ (LOT#)	#INJECTIONS RUN/TOTAL	CASES RUN

Form 426-QC

ANNUAL NIST QC RUN

SCIENTIST:_______________ **QC DATE:**____________

At a minimum of once a year, an 'in-date', certified NIST-SRM standard will be analyzed with our standard procedures. Control or known blood samples may be analyzed simultaneously to 'certify' them for use as NIST QC samples. These samples will be listed in the comments section of this form with lot # and that they were certified. After completion of the QC, the newly 'certified' samples, or their container, will be marked as "NIST Certified" with the corresponding date.

The GeneMapper® ID Data will be analyzed as usual and quality of results will be reflected in the comments or 'passed' areas as appropriate and necessary. Passing results are obtained by achieving the expected results for the given NIST sample(s) and any associated controls. The GeneMapper® ID Electropherograms and an Allele Table will be printed [for the NIST sample(s)] and stored in the Forensic Biology QC binder.

Run Folder: _____________

QC PASSED: **YES** ☐ **NO**☐

Comments: