APPROVED BY:

Biology Program Manager Date: 4/24/2002

Lab Improvement Manager Date: 4-26-2002

INTRODUCTION

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 QA (Quality Assurance) manual, along with the ISP Forensic Services Quality 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 methods, SOPs (Standard Operating Procedures), 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

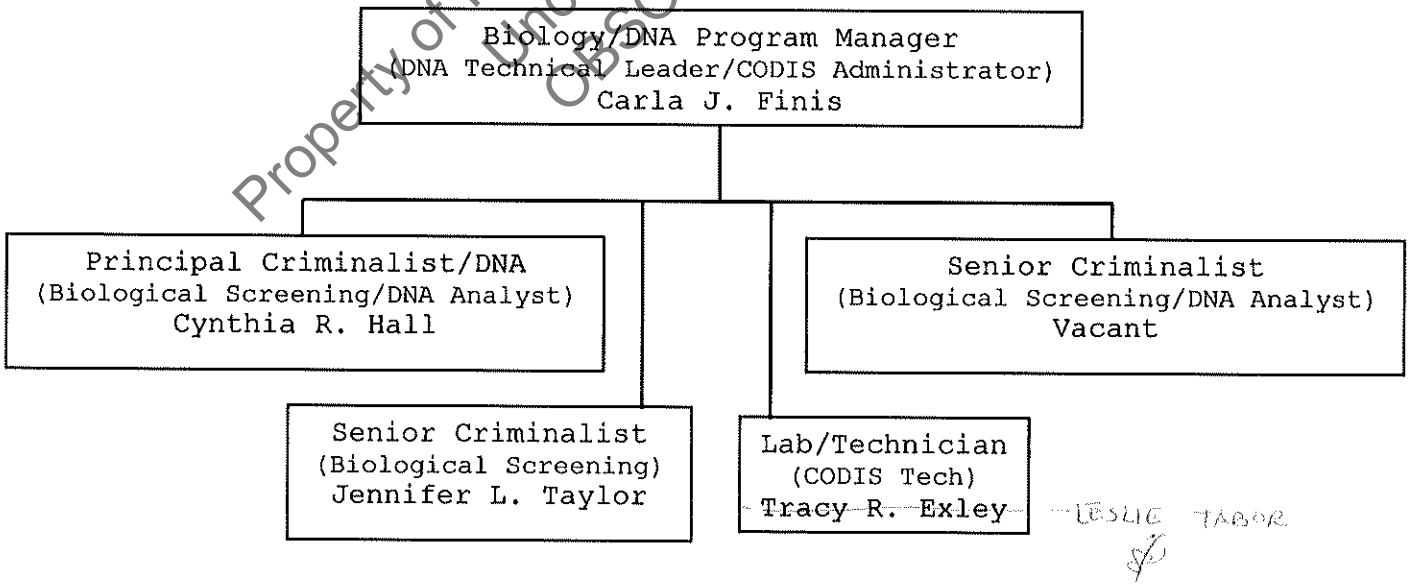
2.1 Organizational Chart and Functional Structure

2.1.1 An organizational chart for ISP Forensic Services appears in the ISP Forensic Services 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 Quality Assurance Standards for 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 5.3 and 5.3 of these documents, respectively). A copy of each may be found in the ISP Forensic Biology Training Manual.



3.0 PERSONNEL QUALIFICATIONS AND TRAINING

3.1 Job Descriptions

Complete job descriptions are available through the Department of Human Resources link on the State of Idaho web site; Biology personnel curriculum vitae are found in this section.

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 Program Manager/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 a Master of Science degree in a biological science. Successful completion of coursework in genetics, biochemistry, molecular biology and statistics.

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).

3.3.1.3 Experience

Must have a minimum of three years forensic DNA laboratory experience.

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 seminars, courses and/or professional meetings.

3.3.2 CODIS Manager

This function may or may not be served by the Forensic Biology Program Manager.

3.3.2.1 Education

Must have a Bachelor of Science in computer science or in a biological science.

3.3.2.2 Training

A combination of training and experience in the use of computers, computer networks, and database systems in a laboratory/scientific setting.

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.

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 a designee) at the annual CODIS State Administrators' meeting. Further educational development to be obtained through relevant courses and/or seminars.

3.3.3 DNA Analyst

The following delineate requirements for a DNA casework or database analyst whose responsibilities include performing genetic analyses on the 310 capillary electrophoresis instruments and data interpretation. DNA extraction 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 a Bachelor of Science in a biological science and successfully completed coursework in genetics, biochemistry, molecular biology and statistics.

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 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 seminars, courses and/or professional meetings.

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 forensic casework, must have a minimum of six months Forensic laboratory experience in the area of Biology/DNA.

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 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.

Jennifer L. Taylor, M.S.

TITLE:	Senior Criminalist
EDUCATION:	B.S., Genetics University of Califonia, at Davis
	M.S., Hazardous Waste Management Idaho State University
CERTIFICATION:	American Board of Criminalistics Diplomate
EXPERIENCE:	1998 - present: Idaho State Police Forensic Services
ADDITIONAL TRAINING:	In-house training: Biological Screening
	In-house training: Courtroom Testimony
	Seminar: Expert Witnesses in the Courtroom - Oregon State Police Forensic Division at Beaverton, OR
	In-service training: Crime Scene Processing - National Crime Investigation & Training at Bannock CSO, Idaho

TRACY R. EXLEY, MPH

TITLE:	Biology Lab/CODIS Technician	
EDUCATION:	B.S., Biology Boise State University	
	Masters', Public Health Idaho State University	
CERTIFICATION:		
EXPERIENCE:	2000 - present:	Idaho State Police Forensic Services
	1998-1999:	Advanced Clinical Research Pharmaceutical Research
	1994-1998:	St. Alphonsus Regional Medical Center Microbiology Section, Laboratory
ADDITIONAL TRAINING:	In-house Forensic Biology Training (Modules 1-3)	
Professional Organizations:		

4.0 FACILITIES

4.1 Laboratory Security

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

4.1.1 Forensic Biology Security

When not under the direct control of Forensic Biology personnel, evidence 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). Only Forensic Biology Personnel will have access to the locked storage. Persons having official business will be allowed access to Forensic Biology only when accompanied by program personnel.

4.1.2 CODIS Security

The CODIS workstation is located in the main Forensic Biology lab 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 the CODIS State Administrator, designated Forensic Biology staff and CJIS personnel will have access to the CODIS Server.

4.1.2.2 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, two weeks of data will be stored offsite.

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

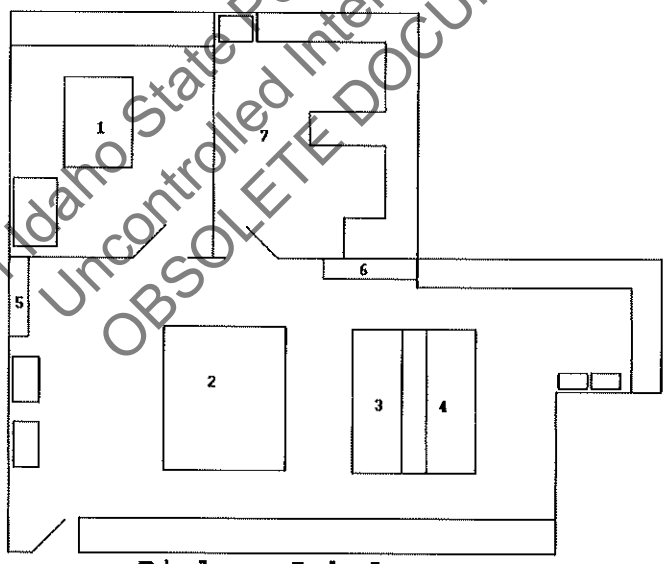
4.1.2.4 The CODIS programs will never be left in a "logged in" state. CODIS users must log in each time they use CODIS and log out prior to leaving the CODIS Workstation.

4.1.2.5 STiMAS, the convicted offender sample tracking database resides on the CODIS workstation and will be accessible, by password, only to Forensic Biology personnel designated by the Program Manager.

4.1.2.6 Personal and identifying information on convicted offenders, which is maintained by hard copy and in the STiMAS database, will be stored separately from the DNA profile (CODIS) information obtained from the genetic analysis of these samples. The DNA profiles will be directly associated only with a unique Idaho Convicted Offender ID number which is assigned by STiMAS upon sample entry.

4.2 Forensic Biology Laboratory Set-up

The Forensic Biology Laboratory is designed to minimize contamination potential during the processing and analysis of evidence. 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.



Biology Lab Areas

1. Primary Evidence Screening
2. Secondary Evidence Screening
3. DNA Extraction
4. DNA Extraction
5. Hood for Bloodstains/Organic Extraction
6. Hood for PCR Amp Set-up
7. Amplification/ Post-Amp Room

5.0 EVIDENCE CONTROL

Evidence which 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 Procedures Manual.

5.2 Forensic Biology Evidence Control

5.2.1 DNA Packet (Sample Retention)

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, a DNA packet is created for each case that is submitted for analysis to Forensic Biology (See BI-102).

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. Notification will consist of a letter sent to the prosecuting attorney informing him/her of possible consumption and requesting the letter be forwarded to defense counsel. This letter will contain a date that testing will commence and allow for either: 1) testing by another accredited laboratory or 2) witnessing (by an acceptable expert) of the sample processing through the set-up of the genetic analysis run. An acceptable expert is a **scientist** with **'hands on'**, **forensic experience** in both the technology used in the analyses, and the corresponding data interpretation. The notification letter will be sent a minimum of 14 days prior to the commencement of testing.

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.

6.0 VALIDATION

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

7.0 Practices and Analytical Procedures

The following is a list of general practices/administrative procedures, analytical methods and forms utilized in Forensic Biology. The remainder of this section consists of those documents.

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
MBI-400 DOCUMENTATION IN FORENSIC BIOLOGY

BI≡Analytical Procedures or Individual Processes

BI-100 PROCESSING LIQUID BLOOD
BI-102 DNA PACKETS
BI-104 PHENOLPHTHALEIN 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-112 BCIP TEST FOR ACID PHOSPHATASE
BI-114 BRENTAMINE TEST FOR ACID PHOSPHATASE
BI-116 SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION
BI-118 SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION
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-202 DNA QUANTIFICATION: QUANTIBLOT™
BI-204 DNA QUANTIFICATION: ACES™
BI-206 DNA QUANTIFICATION: KODAK IMAGE STATION
BI-208 STR AMPLIFICATION: PP16
BI-210 STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS
BI-301 CODIS SAMPLE RECEIPT AND STIMAS ENTRY
BI-310 CODIS SAMPLE EXPUNGEMENT

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

ADD 103 0-TOL

100-BI PHENOLPHTHALEIN REAGENT
102-BI HYDROGEN PEROXIDE 3% (v/v)
104-BI AMMONIUM HYDROXIDE (~3%)
106-BI SODIUM CHLORIDE (NaCℓ) 1M
108-BI OUCHTERLONY DESTAIN
110-BI OUCHTERLONY STAIN
112-BI BCIP REAGENT
114-BI 10X BRENTAMINE (SODIUM ACETATE) BUFFER
116-BI BRENTAMINE SOLUTION A
118-BI BRENTAMINE SOLUTION B
120-BI SALINE (0.85% NaCℓ)
122-BI 10X PHOSPHATE BUFFERED SALINE (PBS)
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)
130-BI NaOH 0.5N
132-BI AMYLASE DIFFUSION BUFFER (pH6.9)
134-BI AMYLASE IODINE REAGENT
136-BI SODIUM HYDROXIDE 5% (w/v)
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)
213-BI TRIS/SODIUM CHLORIDE/EDTA (TNE)
215-BI N-LAUROYLSARCOSINE (SARKOSYL) 20%
217-BI SPERM WASH BUFFER pH7.5
221-BI DTT (0.39M)
223-BI DTT (1M)
225-BI CHELEX REAGENT 5%
227-BI LITHIUM CHLORIDE (LiCℓ) 8M
229-BI PCR-TE (TE^{-4}) BUFFER (10mM TRIS-HCℓ, 0.1M EDTA)
231-BI NaOH 5N
233-BI SODIUM CHLORIDE (NaCℓ) 5M
235-BI SALINE SODIUM CITRATE BUFFER (SSC) 2X
237-BI DENATURATION/SPOTTING SOLUTION (0.5N NaOH, 0.5M NaCℓ)
239-BI NEUTRALIZATION SOLUTIONJ (2X SSC, 0.2M TRIS-HCℓ, pH7.5)
241-BI QUANTIBLOT SPOTTING SOLUTION (QSS) (0.4n NaOH, 25mM EDTA)
243-BI HYBRIDIZATION SOLUTION (5X SSPE, 0.5% SDS)
245-BI QUANTIBLOT WASH SOLUTION (QWS) (1.5X SSPE, 0.5% SDS)
247-BI CITRATE BUFFER pH5, 0.1M (QCB)
249-BI BOVINE SERUM ALBUMIN (BSA) 4%

Moved (in order)

101-BI	BIOLOGY SCREENING SUMMARY
200-BI	DNA EXTRACTION WORKSHEET
202-BI	DIFFERENTIAL DNA EXTRACTION WORKSHEET
204-BI	QUANTIBLOT WORKSHEET
206-BI	ACES WORKSHEET
208-BI	DNA CONCENTRATION WORKSHEET
210-BI	STR AMPLIFICATION SET-UP
212-BI	STR BLIND CONTROL GENOTYPE CHECK
214-BI	STR TECHNICAL REVIEW CHECKLIST
306-BI	STR CODIS REVIEW CHECKLIST
310-BI	CODIS SAMPLE EXPUNGEMENT CHECKLIST
400-QC	FORENSIC BIOLOGY CHEMICAL INVENTORY
402-QC	FORENSIC BIOLOGY EQUIPMENT MAINTENANCE/REPAIR RECORD
404-QC	FORENSIC BIOLOGY WEEKLY QC (**P**ocatello/**C**dA variation)
406-QC	FORENSIC BIOLOGY MONTHLY QC (**P**ocatello/**C**dA variation)
408A-QC	FORENSIC BIOLOGY QUARTERLY QC (N/A for Pocatello/CdA)
408B-QC	FORENSIC BIOLOGY QUARTERLY QC (**P**ocatello/**C**dA variation)
410-QC	QC ABACARD® HEMATRACE® KIT
412-QC	QC ONESTEP ABACARD® P30 KIT
414-QC	PCIAA QC
416-QC	QUANTIBLOT WORKSHEET
418-QC	ACES KIT QC
420-QC	QC STR KITS
422-QC	310 INJECTION LOG
424-QC	MONTHLY 310 QC RUN
426-QC	ANNUAL NIST QC RUN

Delete. Pocatello/CdA references

MBI-100	
8/28/01	8-30-01
	RPS

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:

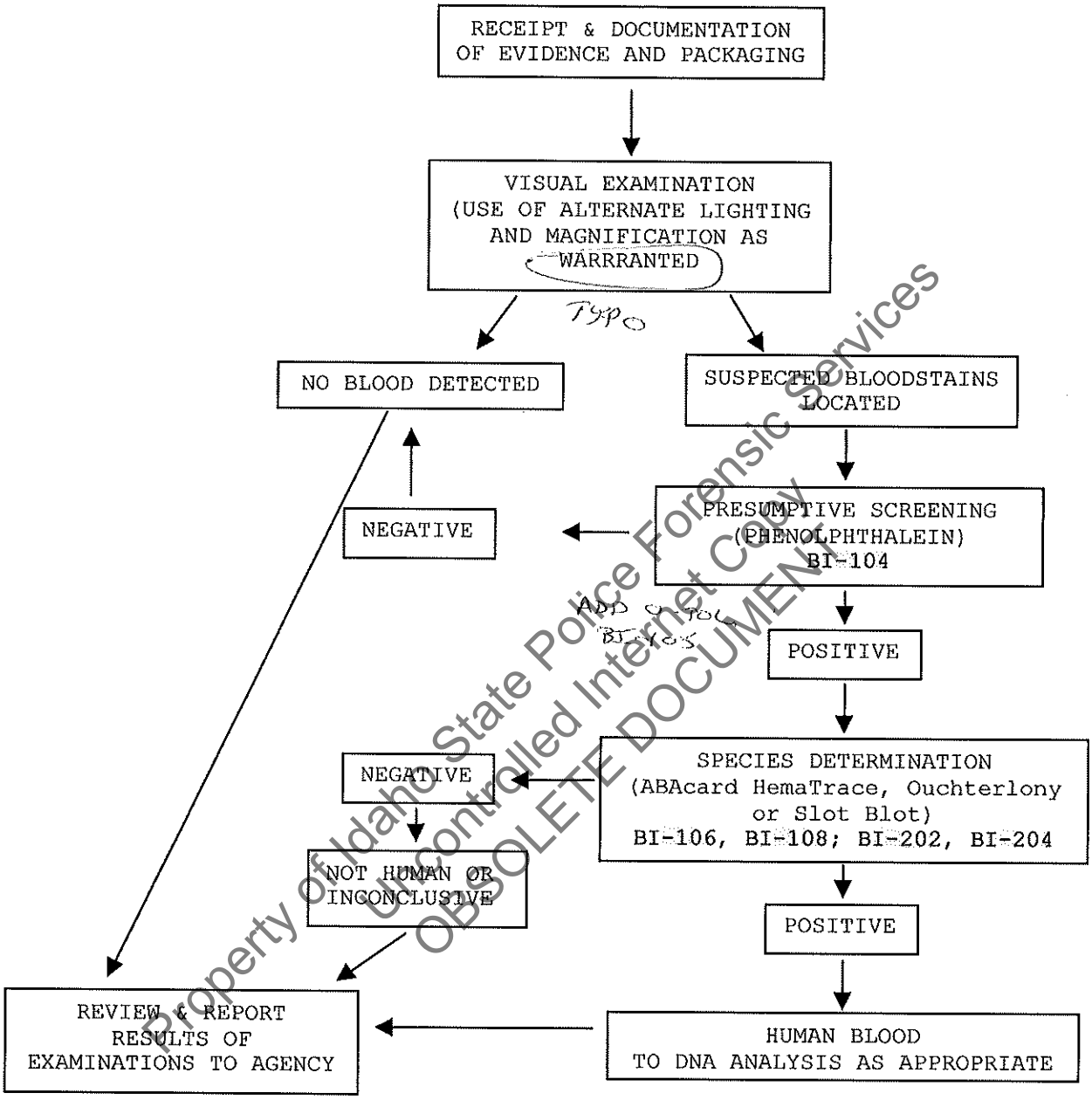
To provide uniform processing of evidentiary material for the presence of blood.

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 Reports of human blood should be 'qualified' as 'probable' in instances where species crossreactivity may be plausible.

MBI-102	
8/28/01	8/30/01

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:

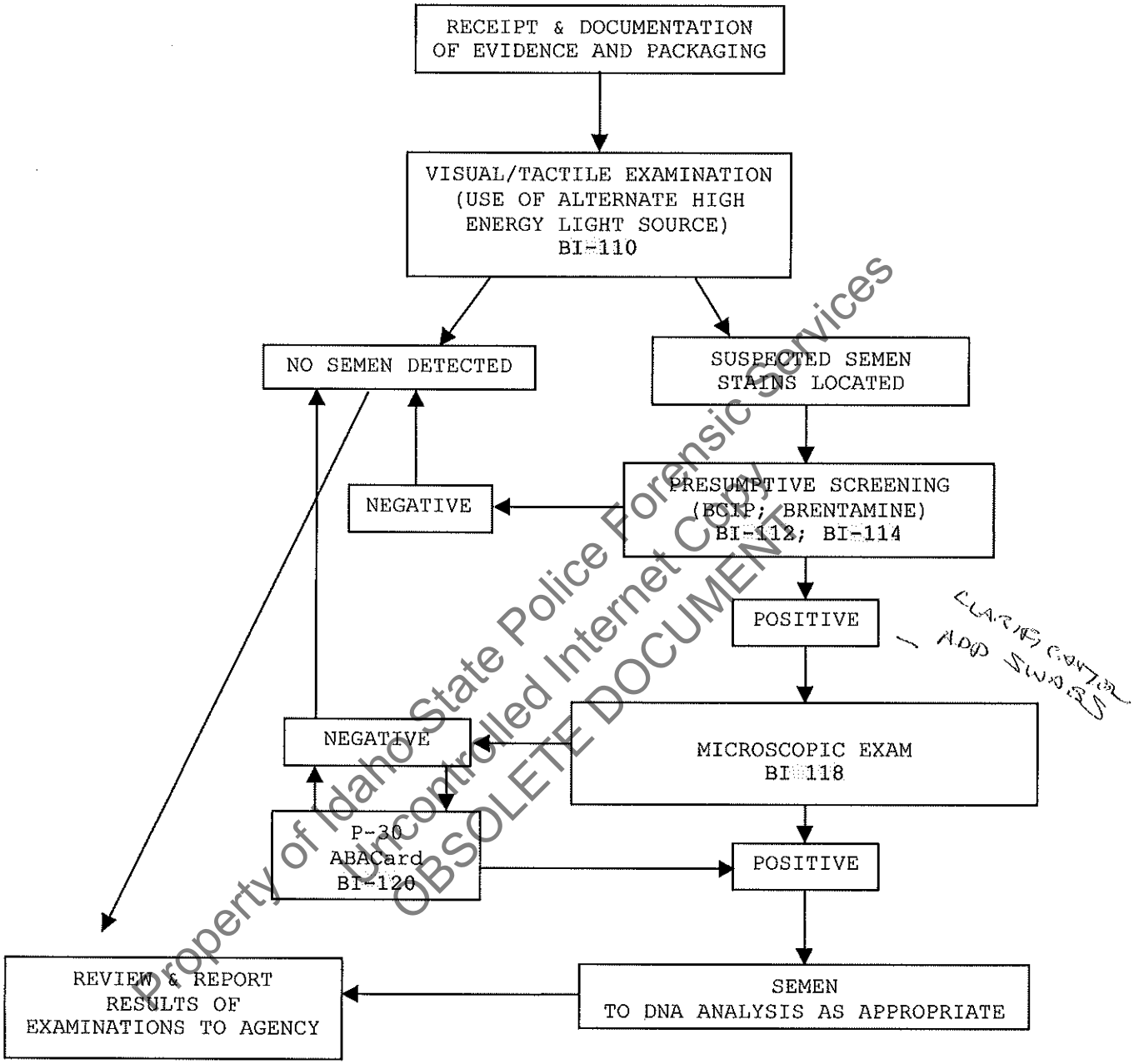
To provide uniform processing of evidentiary material for the presence of semen.

3.0 EQUIPMENT/REAGENTS:

Normal room lighting conditions and the use of an alternative 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 Presumptive AP screening will automatically be performed on pant/panty crotches.

— CLARIFICATION

5.2 A P-30 test need not be performed on vaginal swabs which yielded a positive microscopic exam or if the microscopic exam was negative and sample collection was performed ≥ 48 hours after the alleged assault.

MBI-200	
8/28/01	9.66-1

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 generally 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.

2.0 SCOPE:

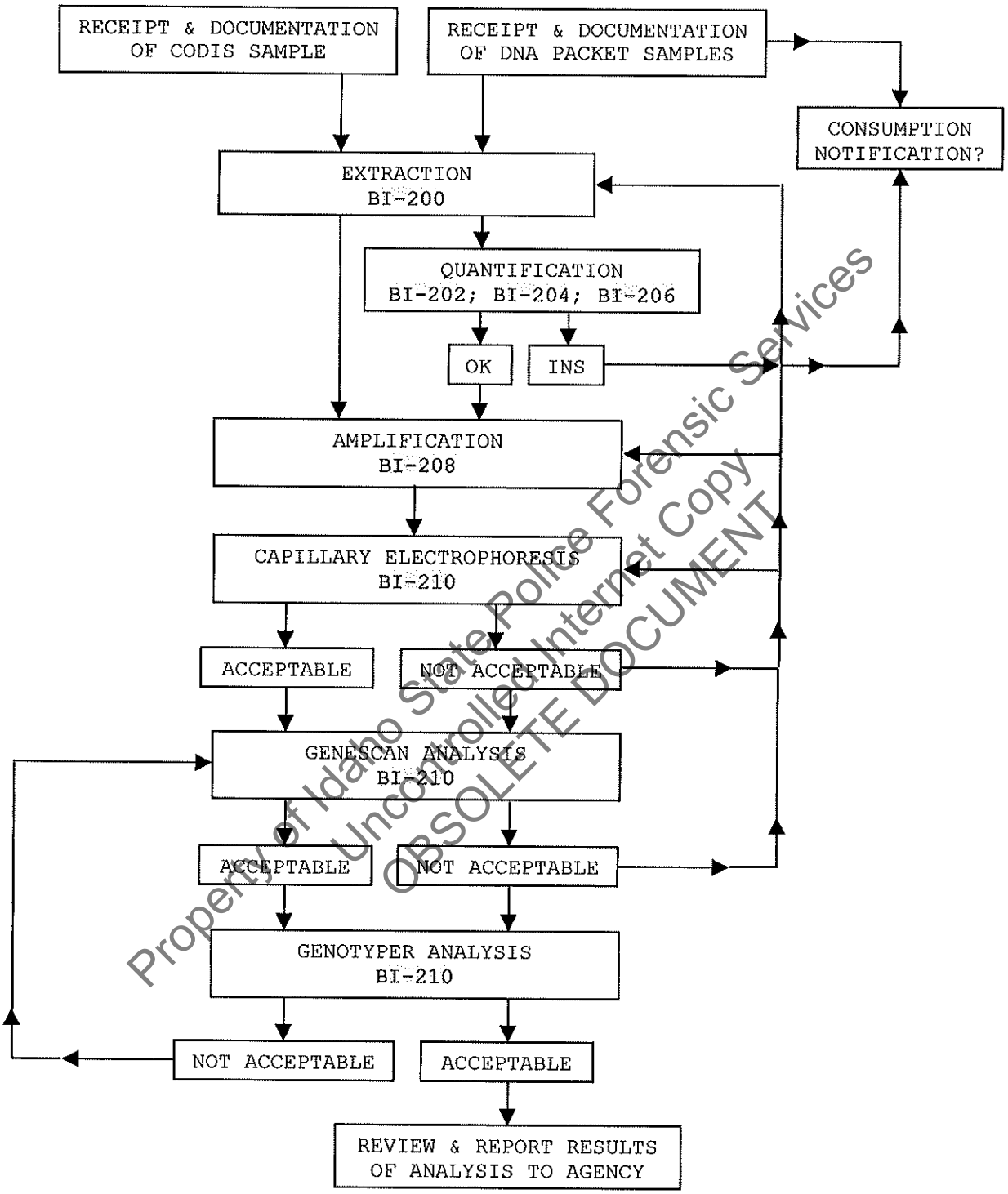
To provide uniform processing of DNA samples to achieve high quality data and consistent interpretation.

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 at the earliest possible point.

reference BI-212

MBI-400	
8/28/01	8/30/01
	RMS

DOCUMENTATION IN FORENSIC BIOLOGY

1.0 BACKGROUND:

Meticulous documentation, particularly for case notes and quality functions is an important aspect of forensic work. Careful reagent and equipment documentation ensures the easiest and most efficient mechanism for tracking problem sources and performing troubleshooting operations. 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.

2.0 SCOPE:

To provide a system of policies, practices, methods and accounting (QC) forms to ensure appropriate documentation is maintained in Forensic Biology.

3.0 EQUIPMENT/REAGENTS:

Various series '400' QC Documents as well as series '100", '200', and '300' analytical procedures and forms.

4.0 PROCEDURE:

4.1 CHEMICALS/REAGENTS

4.1.1 COMMERCIALLY PURCHASED CHEMICALS

4.1.1.1 Biology Personnel should consult the Chemical Inventory Log (Form 400-QC) prior to ordering any new chemical. If the inventory is such that the chemical does need to be ordered, the individual should mark 'ordered MMDDYY' in the 'QUANTITY REMAINING' category to avoid duplicate orders and excess stock.

4.1.1.2 Upon receipt of a chemical or kit, a new entry will be made in the Chemical Inventory log. The scientist should place a single line through the 'ordered MMDDYY' on the chemical's previous entry to indicate that the order has been received. The chemical(s) will be marked with the date received and the individual's initials ('RCVD MMDDYY INITIALS'). If it is an outer container that the chemical/kit remains in until use, the inner container will be labeled with this receipt date when removed for use. Packing slips should be checked to ensure appropriate accounting. 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. 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.msc.cornell.edu/helpful data/msds.html or http://hazard.com/msds/.

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

4.1.2 REAGENTS PREPARED IN-HOUSE

4.1.2.1 All biology reagents will be made with great care, following all quality and safety procedures.

4.1.2.2 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. This form must be filled out. A reagent label must be made that has the lab lot number (note: the lab lot number is designed to incorporate what the reagent is as well), the date, the individual's initials and an expiration date, where appropriate. An expiration date of one year from the date made will be used for reagents without shorter expirations. The labels will have NFPA symbols and these must be filled out by referring to the reagent sheet. 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.

4.1.3 CRITICAL REAGENTS

CRITICAL REAGENTS are those reagents that, if improperly functioning, could result in significant loss or destruction of forensic case or CODIS material and are not amenable (or it's not practical) to testing immediately before the forensic samples with each use. The following reagents have been identified as critical in Forensic Biology. These reagents must undergo a **QC ASSAY BEFORE** use on forensic samples.

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

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

PCIAA (Phenol:Chloroform:IsoAmyl Alcohol; 25:24:1; Form 414-QC)

QuantiBlot® Human DNA Quantitation Kit (Form 416-QC)

ACES™ Human DNA Quantitation System (Form 418-QC)

STR Kit (Form 420-QC)

4.2 EQUIPMENT/INSTRUMENTATION

4.2.1 New purchases, property transfer, and disposition will be tracked on the BIOLOGY EQUIPMENT INVENTORY Spreadsheet. Additional information on the speadsheet includes: property number, description, estimated life expectancy and anticipated replacement date, actual replacement date and a comments section.

4.2.2 OPERATING MANUALS for most equipment/instrumentation will be maintained in the product information file (Manuals for the ABI PRISM™ 310 Genetic Analyzers and Thermalcyclers will be maintained in the Amp/PostAmp Room in close proximity to the instruments).

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

The records for the ABI PRISM™ 310 Genetic Analyzers will be maintained in each instruments QC binder

Any equipment/instrumentation function (not documented on weekly, quarterly, semiannual or annual QC Check lists) will be recorded on the Equipment Maintenance/Repair form (Form 402-QC) and maintained in the Biology QC Binder. Equipment Failure will also be reported on this form.

4.2.4 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 reported on Form 402-QC.

4.2.5 The SCHEDULE of QC Checks is as follows:

WEEKLY (Form 404-QC)

Nanopure Water System Check
Refrigerator/Freezer Temperature Check
Eye Wash Station Check
Water Bath(s) Temperature Check
Heating Block Temperature Check
Amp/Post Amp Temp Chart Check/Replace
Oven Temperature Check

Add RO System

MONTHLY (Form 406-QC)

Microscopes Cleaned → ANNUAL
Pipettes Cleaned
Centrifuges Cleaned
BioSafety Hood(s) Flow Check and Clean
pH Meter High/Low pH Check
PCR Lab Cleaned
Autoclave Clean and Check
Artel PCS2 Calibration Check
STR QC Run (Form 424-QC) → QUARTERLY

QUARTERLY

Thermalcycler Verification Tests (Form 408A-QC)
MacIntosh HD Optimization (Form 408A-QC)
Balance Calibration Check (1g/100mg ±.03g) (Form 408B-QC)
Chemical Shower Check (Form 408B-QC)

ADD CENTRIFUGE ROOM ✓ 408B-QC

SEMIANNUALLY

Matrix for the ABI PRISM™ 310 Genetic Analyzers (The matrix used may or may not be replaced; best will be used) Data will be filed in instrument QC binder (see BI-210).

ANNUALLY

Pipette Calibration Check (Form 402-QC)
Thermalcycler Verification Kit Calibration (Form 402-QC)
Biological Hoods (Form 402-QC)
Digital Temperature Recording Devices (Form 402-QC)
ABI PRISM™ 310 Genetic Analyzers (Form 402-QC)

In addition to the above schedule, personnel should check appropriate parameter function, on all instrumentation, with each use. Any problems noted should be brought to the attention of the necessary supervisory personnel and documented on Form 402-QC.

4.3 FILE DOCUMENTATION, REPORTS AND REVIEW

4.3.1 CASE NOTES

4.3.1.1 Each page of case notes should have the following: Laboratory Case Number, Date, Scientist's Initials and page number (in the form of p. 1 of "X", p 2 of "X" ... p "X" of "X" **or** 1/"X", 2/"X" "X"/"X").

(CONSISTENCY)

4.3.1.2 Case notes are associated with a particular report. If submissions come in for examination after a report(s) has been issued, the page number in the case notes will reflect their association with a supplemental report by the appropriate 'suffix' to the page numbering (e.g., 1s of "X"s; 1s2 of "X"s2 for page numbering in supplemental notes 1 and 2, repectively **or** 1s p 1 of "X"; 2s p 1 of "X").

4.3.1.3 All evidence submitted 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. A description of the evidence should also appear in the case notes with a notation about not being examined at the time, if that's the case. The items should also appear in the "not examined" statement of the report.

4.3.1.4 The description of evidence packaging should be comprehensive and include the type and condition of seal(s). Any differences in the description on the package versus what was entered into ETS (or what the evidence is once opened) should be noted.

4.3.1.5 Every attempt should be made to gain entry into the evidence without breaking any of the original seals. However, if a seal is broken to gain access, it must be noted. Any seal altered or created by a scientist will bear their initials and date across the seal.

4.3.1.6 Evidence descriptions should be "unique" inasmuch as possible (i.e., one pair blue jeans is **NOT** adequate). They should include 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).

4.3.1.7 Evidence numbering MUST be unique for the purpose of possible later CODIS entry. Items should be numbered as follows:

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.

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 delineated as above (e.g., Item 1A-1, Item 1A-2 ...)

4.3.1.8 The Biology Screen Case Summary Form (Form 101-BI) may be useful in summarizing analyses. Any analysis or summary form must be appropriately numbered in the "case notes". 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. Files should be organized from front to back as follows: report, case summary form, chronological case notes/forms, chain of custody, phone/info log (on 'tangerine' paper), and agency materials submitted with evidence. When report has been issued, this documentation should be bound (e.g., stapled) together.

4.3.1.9 Photography, digital or otherwise, is often useful in documenting the appearance of evidence items. However, it is not meant to replace drawing, which on many occasions, is the better choice. Careful drawing and description result in careful and detailed examinations.

4.3.2 REPORTS

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

4.3.2.1 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.

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

(1) CASE SUBMISSION INFORMATION INCLUDING (at a minimum): CASE#, REPORT DATE, SUBMITTING AGENCY, AGENCY CASE#, PRINCIPALS (VICTIM, SUSPECT ETC.), AND OFFENSE DATE.

FORENSIC BIOLOGY REPORT

RESULTS OF EXAMINATION

Statements (see below) regarding evidence exam, results and conclusions. The order of statements will be: 1) positive statements (detection of body fluid), 2) negative statements, 3) inconclusive statements and 4)statements regarding items not examined.

Disposition of Evidence

Statements (See below) regarding evidence retention and return.

Evidence Description

Description of Items submitted for examination.

In first report, all evidence items should be listed (including known reference samples). In supplemental report only those relevant to the additional examinations need to be listed.

(2) I certify that all of the above are true and accurate.

Signature

_____

Name of Scientist
Title of Scientist

[1]If scientist elects to work within ETS, this information will already be there, albeit in a different format. If the scientist prefers to write a report outside of ETS, a standard template has been provided.

[2]This statement is for reports that do not include an attached affidavit.

4.3.2.3 The following statements are to be used in a biology screening report:

Statement Preface:

Examination of the following items: [used for statements regarding > 3 items]

OR

Examination of "item description" (Item#), "item description" (Item#), and "item description" (Item#) [prefaces statements regarding ≤ 3 items]

For definitive positive or negative statements:

"Preface see above" detected (or did not detect) the presence of (semen, blood, human blood, non-human blood or saliva).

For 'Qualified' Statements:

"Preface see above" indicated the presence of blood; however, tests for confirmation, and to determine the species of origin, were inconclusive.

"Preface see above" detected semen by the presence of the semen specific protein, p30; however, no spermatozoa were observed.

Testing for amylase, an enzyme found in saliva, was performed on "Preface see above". The results (indicate the possible presence of saliva **OR** were inconclusive for the presence of saliva see BI-122 or BI-124 for explanation).

For items not examined (all items not examined will be included; reference samples do not fall into this category):

"Preface see above" were not examined at this time.

delete

For reports in cases where the status of a DNA testing request is not known:

If additional testing is desired, please contact the Laboratory regarding the request PRIOR to resubmission.

For reports in cases where it is known that DNA analysis is going to be performed:

The following samples have been forwarded for DNA analysis: [list as in Preface see above and include known bloodstains from "name" (Item#)]. Results will follow in a separate report.

For reports in cases where DNA analysis has been requested but known reference samples have not been submitted (i.e., the victim sample or a suspect or elimination sample in a case where there is an **'IDENTIFIED SUSPECT'** -to distinguish the nonsuspect case which will be processed for CODIS entry):

DNA testing can be performed upon submission of a known reference sample(s) from [list name(s)]. Results will follow in a separate report.

Evidence Disposition Statement:

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

Evidence Description 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 containing "description", *(include the following if collection information is known)* said to have been collected from "name" or "location".

A tape-sealed evidence submission envelope containing "description", *(include the following if collection information is known)* said to have been collected from "name" or "location".

4.2.3.4 The following statements are to be used in an STR DNA Report:

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

For statements regarding profile matches that have met the 'source attribution' criterion (estimated frequency in population of less than 1 in $1.6x10^{10}$):

[1]The DNA profile obtained from the "item description (Item #)" matches that obtained from the blood sample of "name". Therefore, "name" is the source of the "(DNA, blood, semen, saliva etc.) ".

For statements regarding profile matches that do not meet the 'source attribution' criterion (estimated frequency in population of greater than 1 in $1.6x10^{10}$):

The DNA profile obtained from the "item description (Item #)" matches that obtained from the blood 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) frequency estimate".

For cases in which consistent profiles were obtained on multiple items, however, data from fewer loci were obtained (partial profile) on some items, the appropriate statement above will be made for the most complete profile(s) and the following statement will be made regarding the additional partial profile(s):

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

For mixture cases:

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 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 this 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"

For statements regarding exclusionary data:

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.)".

For statement regarding items in which no DNA profile was obtained:

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

The following footnote will appear at the bottom of any report page that has a 'source attribution' statement:

[1]This conclusion is based upon a genetic match at the gender identity locus, Amelogenin, in addition to the following "number" polymorphic STR loci that have an expected population frequency of less than 1 in "actual (most conservative) frequency estimate". It also assumes that "name" does not have a genetically identical twin.

On each report there will be a statement regarding the loci examined:

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.

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.

The Evidence Disposition Statements, Evidence Description, certification, and signature block follow the same format as in the Biology Screening Report format above.

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.

4.3.3 REVIEW

4.3.3.1 100% of the examinations and reports documented and/or issued from Forensic Biology will be "peer-reviewed".

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

4.3.3.3 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).

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

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

4.3.3.6 The second scientist will also place their initials below the signature of the scientist issuing the report (the scientist will also make the appropriate notation on the case file jacket).

BI-100	
8/28/01	8/31/01

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 tube. In a liquid state, these samples are more susceptible to degradation resulting in the loss of DNA. Bloodstains stored in a dry state, even at room temperature, may be suitable for DNA testing for many years.

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

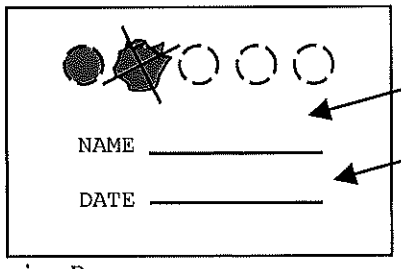
Schleicher & Schuell: Blood Collect Card(s) #903
Sterile, Disposable Transfer pipet
or 1 mℓ pipet with sterile tip

4.0 PROCEDURE:

4.1 Label stain card with Case Number and Item Number (in place of "NAME"), Date and Initials (see below).

4.2 Take tube containing blood sample and mix thoroughly by inversion.

4.3 Remove cap from blood tube and draw ~1mℓ of blood into pipet. Carefully spot blood onto circle, filling, but not over saturating, on stain card. Repeat with the four remaining circles on the card (see below).



Case Number and Item Number (e.g., M20011234 It. 1A)

Preparer's Initials

clarification see 5.3 note

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

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

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 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.

ADD 5.3 clarification of card labeling

BI-102	
8/25/01	8/30/01

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, a DNA packet is created for each case that is submitted for analysis to Forensic Biology.

2.0 SCOPE:

To provide a method to ensure adequate sample retention for sample re-analyses and new protocol/technology development. DNA Packet creation is generally initiated when processing the known reference blood samples from principals in a given case (See BI-101).

Add "where possible"

3.0 EQUIPMENT/REAGENTS:

Schleicher & Schuell: Blood Collect Card(s) #903
Coin Envelopes (~3⅛" x 5½")
DNA Packet Envelope (~6½" x 9¼" manila envelope)

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. Each coin envelope will be labeled with Case Number, Item Number, Date, Scientist's Initials and sealed with evidence tape.

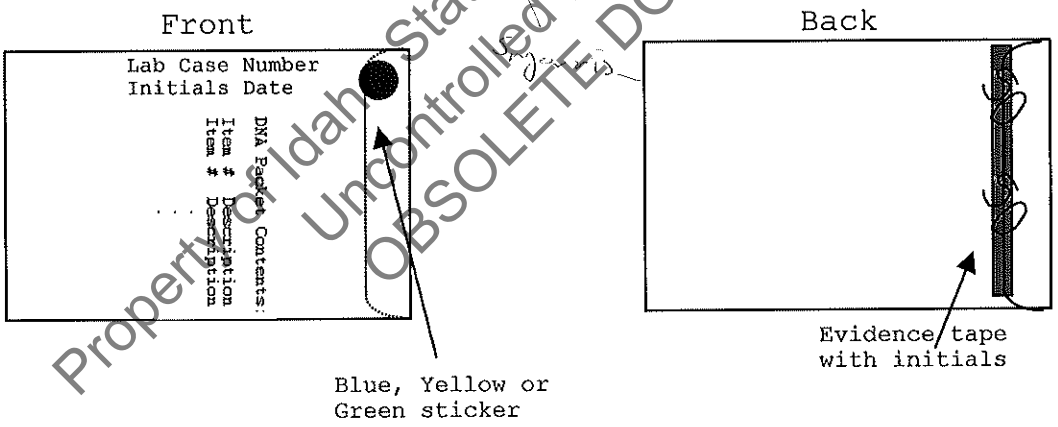
4.2 All sealed coin envelopes will be placed inside a larger manila envelope (DNA Packet Envelope) and labeled as below.

4.3 The DNA packet itself will 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) 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) 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 TRS and entered as an additional item of evidence ("DNA Packet containing ...") to allow for tracking in the ETS. The storage location will have a barcode.

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



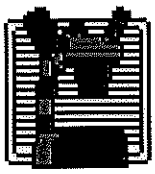
4.7 Following DNA testing, any leftover DNA extracts will be sealed (with parafilm or other sealant to prevent leakage and/or evaporation) and placed in the DNA Packet.

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, the individual DNA Packets should still be entered into ETS as a single evidence item but labeled "1 of X, 2 of X ...X of X".



BI-104	
8/28/01	8/30/01

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:

Phenolphthalin Stock
Ethanol
3% Hydrogen Peroxide
Sterile Cotton Swabs

4.0 PROCEDURE:

4.1 Positive (know bloodstain) and negative (sterile/nanopure H_2O) control samples are processed and determined to be working properly prior to testing any forensic samples.

4.2 Sterile cotton swabs are used to collect the suspected blood onto the tip of a swab. The swab may be moistened with sterile/nanopure H_2O if necessary.

4.3 To the swab with the suspected blood, add 1-2 drops of Ethanol, followed by 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).

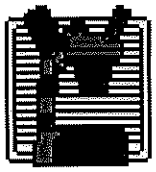
4.5 Document result in case notes. Record positive (+) or negatives (-). Analyst may use other descriptive word(s) as well (e.g., strong, weak,).

5.0 COMMENTS:

5.1 A piece of filter paper may also be used for collection and testing of suspected bloodstains.

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

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



BI-106	
8/28/01	8/30/01
	RPL

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, saline, 1XPBS or PCR-TE.

ADD VOL DS for SAMPLE CONSERVATION *

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, skunk). Therefore, reports of human blood should be 'qualified' as 'probable' in instances where species crossreactivity may be plausible. It should be noted that crossreactivity with nonprimate species may be eliminated by reducing the size of the sample to be extracted (e.g., a single 2mm thread).

BI-112	
8/28/01	8/30/01
	NDG

BCIP TEST FOR ACID PHOSPHATASE

1.0 BACKGROUND:

Prostatic 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.

Baechtel, F.S., Brown, J., and Terrell, L.D. 1987. Presumptive Screening of Suspected Semen Stains *In Situ* Using Cotton Swabs and Bromochloroindoyl Phosphate to Detect Prostatic Acid Phosphatase Activity. *Journal of Forensic Sciences*, 32: 880-887.

2.0 SCOPE:

To provide a rapid screening method for the localization and presumptive identification of semen on evidentiary items.

3.0 EQUIPMENT/REAGENTS:

BCIP Reagent
Oven or Water Bath

4.0 PROCEDURE:

4.1 Label test tubes for suspected semen stains and controls.

4.2 Lightly rub a known semen stain with a sterile, pre-moistened cotton swab and place swab in appropriately labeled test tube. Repeat process for suspected semen stains (or apply 10µl extract where appropriate) and use a pre-moistened swab for a negative control.

10-20 µl — 50-100 µl extract
depending on

4.3 Add sufficient BCIP reagent to each test tube to cover the cotton tip.

4.4 Incubate for 15-20 minutes at 37°C.

4.5 While positive results, visualized by the development of an aqua color, may occur and be recorded in less than 5 minutes, a negative result may only be recorded after a full 20-minute incubation. Results are recorded as positive (+) or negative (-) and additional grading (e.g., 1^{+}-4^{+}) or description (strong, weak, delayed) may also be used.

5.0 COMMENTS:

5.1 Upon aging, the BCIP reagent will develop an aqua color and should be discarded at the point at which it could interfere with the interpretation of the negative control.

5.2 To avoid false positives, the incubation time should NOT be extended beyond 20 minutes. False positives are generally, delayed, weak reactions that may appear on the swab only and not appreciably change the color of the BCIP reagent itself.

BI-114	
9/21/01	10-3-01
	RPS

BRENTAMINE TEST FOR ACID PHOSPHATASE

1.0 BACKGROUND:

Prostatic 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:

10X Sodium Acetate Buffer
Brentamine Solution A
Brentamine Solution B

4.0 PROCEDURE:

4.1 Prepare Brentamine Working Stock : Mix 1 part solution A and one part solution B with 8 parts of water (or use pre-made mix per manufacturer's instructions). This solution should be prepared fresh each day it is used.

4.2 Lightly rub a known semen stain with a sterile, pre-moistened cotton swab. Repeat process for suspected semen stains (keeping samples well separated; e.g., in rack or test tubes) and use a pre-moistened swab for a negative control.

4.3 Add Brentamine Working stock to sample(s).

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

5.0 COMMENTS:

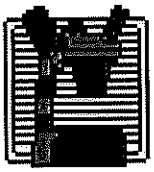
5.1 Upon aging, the Fast Blue B Salt solution develops a purple color and may interfere with weak result interpretation. The reagent must be discarded when this color affects the negative control.

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

5.3 Test may also be performed using 10µl of a sample extract.

5.4 This test may also be used for mapping, 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. A known semen stain is pressed against the paper (in a non-evidence area) to serve as a positive control. The paper is sprayed with Brentamine Reagent and analyzed as above.

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



BI-116	
8/28/01	10-3-01
	RBE

SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION

1.0 BACKGROUND:

The identification of semen is a multi-step process for which it is necessary to generate both liquid and solid 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 and confirmatory testing for the presence of semen as well as other forensic analyses.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 Label tubes with identifying information.

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

ADDL VOL OPTION

4.3 Remove the substrate and spin the extracts for ~5 minutes at low speed (~2,000-3,500 rpm).

4.4 Carefully remove all but ~30-50µℓ of supernatant without disturbing the pellet. The supernatant will typically be used for AP screening (BI-112; BI-114) and p30 detection (BI-120), while the remaining sample will be resuspended and used for microscopic examination (BI-118).

5.0 COMMENTS:

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

BI-120	
8/28/01	8/30/01

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

1.0 BACKGROUND:

P-30 is a seminal-fliud-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 and control extract (see BI-116). to ~190-200µℓ (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 Both positive (known semen stain extract or Seri™ semen standard [10ng; 10µℓ of a 1:100 dilution]) and negative (saline) controls are used.

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

5.4 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.

5.5 As with any antigen/antibody interaction, excess antigen may lead to a 'high dose' 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.

BI-200	
8/28/01	9/04/01
	NDE

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 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:

Centricon® Microconcentrator Devices
Microcentrifuge
15/50mℓ conical tubes
37/56/65°C Water Bath or Other Incubator
Fixed Angle Centrifuge
1.5mℓ microcentrifuge Tubes (1.5mℓ tubes)
MicroAmp Tubes
Coarse Sandpaper

4.0 REAGENTS:

Stain Extraction Buffer (SEB)
PCR TE (TE, 10mM Tris-HCl; 0.1mM EDTA, pH 8.0)
Tris/EDTA/Sodium Chloride (TNE)
20% Sarkosyl
Proteinase K (ProK, 20 mg/mL)
0.39M and 1M Dithiothreitol (DTT)
Sperm Wash Buffer (SWB)
Phenol/Chloroform/Isoamyl Alcohol (PCIAA, 25:24:1)
Absolute Ethanol (Abs EtOH)
Phosphate Buffered Saline (PBS)
Ethyl Ether
Xylene
Liquid Nitrogen
Dry Ice
FTA Purification Reagent
8M LiCℓ

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 must be set up first.

Caution: See Comments 1 and 4.

5.1 BLOODSTAINS AND SALIVA SAMPLES: (ADDED OTHER NON-SEXUAL ASSAULT SAMPLES

5.1.1 For stains on cloth or porous materials, Add an ~3mm^2 cutting/sample to a sterile 1.5mℓ tube (for smaller stains consume sample). For stains deposited on non-porous objects, collect sample using a sterile cotton-tipped applicator with a small amount of sterile deionized water, TE or SEB. Using a clean scalpel, cut a swab portion to yield an equivalent stain amount (~3mm^2) and place it into a sterile 1.5mℓ tube.

5.1.1a 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 **5.1.2**.

5.1.2 Add the following to the tube:
400µℓ SEB
10µℓ Pro K

5.1.3 Vortex briefly and incubate at 56°C for a minimum of 3 hours (may be left overnight).

5.1.3a Optional: For most stains (i.e., ~3mm^2) the cuttings/substrate will not interfere with organic extraction and need not be removed prior to the addition of PCIAA. Larger cuttings/samples can be removed either by piggyback centrifugation (invert sample tube and use sterile pushpin or needle to poke hole in bottom of tube, place upright inside new sterile 1.5mℓ tube) or by using a spin basket. Spin in centrifuge at low speed (3,000-5,000 rpm) for ~5 minutes to remove substrate. Proceed to **5.1.4.**

5.1.4 In a fume hood, add 500µℓ phenol/chloroform/isoamyl alcohol to the stain extract. Mix vigorously by hand to achieve a milky emulsion. Spin in microcentrifuge for 2 minutes to achieve layer separation.

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or microconcentration (See **6.0**).

5.2 DIFFERENTIAL EXTRACTION OF SEMEN-CONTAINING SAMPLES:

Note: For removal of sample from mounted slide, see **8.0.**

5.2.1 Add cutting/sample (the size of sample used will be case-dependent and based upon microscopic exam and total sample amount) to a sterile 1.5mℓ tube and add:

400µℓ TNE
25µℓ 20% Sarkosyl
75µℓ sterile H_2O
10µℓ Pro K

5.2.2 Incubate at 37°C for ~2-3 hours. Sonicate the sample briefly (≤ 10 seconds) after ~1 hour and again after the completion of the incubation.

5.2.3 Mark a new sterile 1.5mℓ tube. Remove substrate by using piggyback or spin basket centrifugation (see **5.1.3a**).

5.2.4 Centrifuge the new tube in a microcentrifuge on high speed (>10,000 rpm) for ≥1 minute to further solidify the pellet.

5.2.5 Remove the supernatant taking care not to disrupt the cell pellet in the bottom of the tube. Transfer this supernatant (♀/epithelial cell fraction) to a new sterile tube and store at 4°C or proceed directly to **5.2.11**.

5.2.6 The purpose of a differential extraction is, typically, to obtain a male fraction that is void of any ♀ contribution. The initial extraction (**5.2.1-5.2.2**), while sufficient to obtain a satisfactory ♀/epithelial cell fraction, is generally not sufficient to remove all of the ♀ contribution. In addition to the sperm cell washes that follow, the initial extraction (**5.2.1-5.2.2**) may, at the scientist's discretion, be repeated 1-2 times at this point. The incubation time may also be increased (up to overnight for a single incubation). This may be particularly useful in instances in which there is an overwhelming proportion of epithelial cells with the presence of sperm that appear intact microscopically. Following the final incubation, the extract should be transferred to a new sterile 1.5mℓ tube and spun as in **5.2.4**. These additional supernatants do not need to be retained.

5.2.7 Wash the sperm pellet as follows: Resuspend the pellet in 500µℓ of Sperm Wash Buffer. Vortex briefly. Spin in a microcentrifuge for ~5 minutes at maximum speed (>10,000rpm). Remove all but ~50µℓ of the supernatant and discard it.

5.2.8 Repeat **5.2.7** ≥1 more time(s). In instances of low sperm amounts, the centrifugation time after each wash may be increased (up to ≤5 minutes) to ensure that all sperm cells remain in the pellet. Alternatively, fewer washes with repeated ♀ extraction incubations may be preferential.

5.2.9 To the remaining sperm pellet solution add:

150µℓ TNE
50µℓ 20% Sarkosyl
40µℓ 0.39M DTT
150µℓ sterile H2O
10µℓ ProK

5.2.10 Mix and incubate at 37°C for a minimum of 3 hours (may be left overnight).

5.2.11 In a fume hood, add 500µℓ phenol/chloroform/isoamyl alcohol to the extract. Mix vigorously by hand to achieve a milky emulsion. Microcentrifuge for 2-3 minutes to achieve layer separation.

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or microconcentration (See **6.0**).

5.3 EXTRACTION FROM HAIR:

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

5.3.1 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) on the hair shaft. If the presence of any body fluid is noted, it may be removed for separate DNA analysis 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 **5.1.1**). Proceed to **5.3.2** with the hair(s).

5.3.2 Once a suitable hair(s), preferably anagen, has been identified it needs to be washed to reduce surface dirt and contaminants. This may be accomplished by immersing the hair(s) in sterile, deionized water in a sterile, 50-mℓ conical tube. 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.

5.3.3 Although the hair(s) was washed 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 is processed separately as a control. The remaining shaft may be retained for subsequent analyses (e.g., trace, mitochondrial DNA).

5.3.4 To a 1.5mℓ tube Add:

500µℓ SEB
20µℓ 1M DTT
10µℓ ProK

Add the hair sample to the tube. Incubate at 56°C for minimum of 6-8 hours (may be left overnight). Hair will usually have softened at this point but may not be completely dissolved. Vortex 30 seconds and proceed to **5.3.5.**

5.3.5 If the hair is completely dissolved proceed to **5.3.6.** If the hair is not completely dissolved add:

20µℓ 1M DTT
10µℓ ProK

Incubate at 56°C for ≥6 hours or until hair is completely dissolved. Vortex 30 seconds.

5.3.6 Spin for 1 minute at high speed at room temperature to remove pigment and particles. Transfer the supernatant to a new sterile 1.5mℓ tube.

5.3.7 In a fume hood, add 500µℓ PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in microcentrifuge for 2-3 minutes at high speed to achieve layer separation.

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or microconcentration (See **6.0**).

5.4 EXTRACTION FROM SALIVA (ENVELOPE FLAPS AND STAMPS):

5.4.1 Cut an ~1.0cm^2 section into small pieces and place in a 1.5mℓ tube. Add 1.0mℓ of sterile water and incubate at 4°C for ≥5 hours (may be left overnight). ADD USE OF STAMM

5.4.2 Mark a new sterile 1.5mℓ tube. Remove substrate by using piggyback or spin basket centrifugation (see **5.1.3a**).

5.4.3 Spin the new tube in a microcentrifuge on high speed (>10,000 rpm) for ≥3 minutes to further solidify the pellet.

5.4.4 Remove the supernatant taking care not to disrupt the cell pellet in the bottom of the tube. Discard the supernatant and retain the pellet for extraction.

5.4.5 To the pellet, add:

400µℓ SEB
10µℓ ProK

Incubate overnight at 56°C.

5.4.6 In a fume hood, add 500µℓ phenol/chloroform/isoamyl alcohol to the extract. Mix vigorously by hand to achieve a milky emulsion. Microcentrifuge for 2-3 minutes to achieve layer separation.

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or microconcentration (See **6.0**).

5.5 EXTRACTION OF DNA FROM SALIVA (CIGARETTE BUTTS):

5.5.1 Using a new, sterile scalpel blade, slice the paper (~1cm wide strip) near (starting ~2.3mm from end) the butt end in an area likely to have had contact with the smoker's mouth.

5.5.2 Remove ~1/2 of the paper slice from the cigarette butt, cut it into smaller pieces, and put the pieces into a sterile 1.5mℓ tube.

5.5.3 To the 1.5mℓ tube, add:

400µℓ SEB
10µℓ ProK

Vortex briefly. Incubate at 56°C for a minimum of 3 hours (may be left overnight).

5.5.4 Mark a new sterile 1.5mℓ tube. Remove substrate by using piggyback or spin basket centrifugation (see **5.1.3a**).

5.5.5 In a fume hood, add 500µℓ PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in a microcentrifuge for 2-3 minutes to achieve layer separation.

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or microconcentration (See **6.0**).

5.6 EXTRACTION FROM FRESH BONES (~1 YEAR OR LESS) AND TEETH:

Caution: See Comments 4.

5.6.1 Obtain a fragment of bone (~ 0.1 gram) and remove tissue using ethyl ether (shake vigorously with a few mℓs of ether in a 15-mℓ polypropylene tube).

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 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 with mortar and pestle or blender (chisel, hammer and press may be used initially). Note: This is best performed using a pre-chilled mortar and pestle. Place the pre-chilled mortar on a bed of dry ice (in a styrofoam box) to keep the evaporation of liquid nitrogen to a minimum. Add a small amount of $liqN_2$ and crush to as fine a powder as possible, adding more $liqN_2$ as needed. Eventual transfer to a 1.5mℓ tube is best accomplished in a step-wise fashion. While frozen, the powder shouldn't stick to the mortar. Slowly swirl to collect powder centrally in the $liqN_2$, tilt mortar toward pouring spout and slowly transfer to a 50-mℓ polypropylene tube. Allow the $liqN_2$ to evaporate completely.

5.6.5 To the tube, add:

400µℓ SEB
10µℓ ProK

Mix thoroughly by hand and transfer extract (using a 1mℓ pipette, with tip end cut-off, or large bore transfer pipette) to a 1.5mℓ microcentrifuge tube 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 2-3 minutes to achieve layer separation. Transfer aqueous layer to new sterile tube. Repeat this step until the interface is clean.

5.6.7 Add 15µℓ 8M LiCℓ and mix by inversion. Add 700µℓ cold absolute ethanol and mix. Precipitate overnight at -20°C.

5.6.8 Spin in microcentrifuge on high speed (>10,000 rpm) for 30 minutes. Gently decant supernatant.

5.6.9 Gently wash pellet with 1mℓ 70% ethanol. Spin in microcentrifuge at high speed (>10,000 rpm) for 10 minutes. Gently decant supernatant and allow pellet to air dry.

5.6.10 Resolubilize the pellet by adding 50µℓ TE and incubating at 56°C for ≥ 1 hour.

5.6.11 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

5.7 EXTRACTION OF DNA FROM AGED BONES:

Caution: See Comments 4.

5.7.1 Obtain a fragment of bone (~2.0 grams) and remove outer layer by sanding.

5.7.2 Rinse bone with sterile, deionized water (shake vigorously with a few mℓs of water in a 15-mℓ conical tube).

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

5.7.4 Crush bone into small pieces with mortar and pestle or blender (chisel, hammer and press may be used initially). Note: This is best performed using a pre-chilled mortar and pestle. Place the pre-chill mortar on a bed of dry ice (in a styrofoam box) to keep the evaporation of liquid nitrogen to a minimum. Add a small amount of $liqN_2$ and crush to as fine a powder as possible, adding more $liqN_2$ as needed. Eventual transfer to a 1.5mℓ tube is best accomplished in a step-wise fashion. While frozen, the powder shouldn't stick to the mortar. Slowly swirl to collect powder centrally in the $liqN_2$, tilt mortar toward pouring spout and slowly transfer to a 50-mℓ polypropylene tube. Allow the $liqN_2$ to evaporate completely.

5.7.5 To the tube, add:

3mℓ SEB
100µℓ ProK

Mix thoroughly by hand and transfer extract (using a 1mℓ pipette, with tip end cut-off, or large bore transfer pipette) to a 15mℓ polypropylene tube and incubate at 56°C overnight.

5.7.6 Add an additional 100µℓ ProK and incubate at 56°C for ≥3 hours.

5.7.7 In a fume hood, add 3.2mℓ PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in a microcentrifuge for 2-3 minutes to achieve layer separation. Transfer aqueous layer to new sterile tube. Repeat this step until the interface is clean.

5.7.8 Add 100µℓ 8M LiCℓ and mix by inversion. Add 7mℓ cold absolute ethanol, mix. Incubate overnight at -20°C.

5.7.9 Transfer 1.5mℓ of solution to a sterile 1.5mℓ microcentrifuge tube. Spin in microcentrifuge on high speed (>10,00 rpm) for ~15 minutes. Gently decant supernatant. Continue to transfer additional 1.5 mℓ of cold sample to the same tube, centrifuge, and discard supernatant until the entire sample has been consolidated.

5.7.10 Gently wash pellet with 1mℓ 70% ethanol. Centrifuge at high speed (>10,000 rpm) for 10 minutes. Gently decant supernatant and allow pellet to air dry.

5.7.11 Resolubilize the pellet by adding 50µℓ PCR TE and incubating at 56°C for ≥ 1 hour.

5.7.12 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

5.8 EXTRACTION FROM BLOOD/SALIVA ON FTA CARDS:

Note: Since the DNA remains bound to the FTA card, regular pipette tips are used throughout and a single tip may be used for each reagent.

5.8.1 Remove a "top punch" (results in the top layer of FTA paper being retain in punch, this is accomplished by placing punch firmly on card and twisting 1/2 turn clockwise and 1/2 turn counterclockwise) sample from FTA card using a 1.2mm Harris punch. Eject sample(s) into microAmp tube(s).

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

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

5.8.4 Repeat **5.8.2-5.8.3** twice.

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

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

5.8.7 Repeat **5.8.5-5.8.6** twice.

5.8.8 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.

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

6.0 DNA ISOLATION PROCEDURES:

Two methods are available for isolating DNA, ethanol precipitation and using a microconcentrator device. It is the scientists' decision, based on training and experience, which method to use. Generally, ethanol precipitation is used for samples in which sufficient DNA is present (e.g., known blood samples). The microconcentrator is used for samples with limited amounts of DNA present (e.g., envelope flaps). ADDED CHANGE samples processed separately where necessary

6.1 ETHANOL PRECIPITATION:

6.1.1 Transfer the aqueous layer, taking care not to disrupt the interface, to a new sterile tube. Add 1.0mℓ of cold absolute ethanol and mix by inversion. Discard the phenol mixture into the organic waste container in the hood. Discard the tube into a biohazard waste container.

6.1.2 Precipitate the DNA at -20°to -30°C for a minimum of 30 minutes (sample may be left in freezer for an extended period of time).

6.1.3 Remove tube from freezer and spin in microcentrifuge on high speed (>10,000 rpm) for ~15-30 minutes.

6.1.4 Remove the ethanol by decantation.

6.1.5 Add 1.0mℓ 70% ethanol, mix by hand, and centrifuge on high speed (>10,000 rpm) for ≥5 minutes. Decant the ethanol and allow sample to air dry.

6.1.6 Resolubilize the DNA in ~50-100µℓ of TE (typically 50µℓ for forensic unknowns and 100µℓ for reference samples or forensic samples expected to contain a great deal of DNA; e.g., ♀ fractions) at 56°C for a minimum of 30 minutes.

6.1.7 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

6.2 ISOLATION VIA MICROCONCENTRATOR DEVICE:

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

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

6.2.3 Add the entire aqueous layer (approximately 400µℓ) to the upper reservoir. Discard the phenol mixture into the organic waste container in the hood. Discard the tube into a biohazard waste container.

6.2.4 Cover the Centricon tube with the retentate cup. Spin in a fixed angle centrifuge at ~2500-3000 rpm for 10-20 minutes. The DNA sample will be concentrated in ~20-40µℓ of TE in the upper Centricon reservoir. Discard the effluent that has collected in the lower reservoir.

Note: The Centricon units are sensitive to rotor forces. Do not centrifuge above 1000 x g. Centrifugation time can be increased if the volume does not reduce to ≤40µℓ in the specified 20 minutes.

6.2.5 Add 2mℓ of PCR TE to the concentrated DNA solution in the upper Centricon reservoir and repeat the centrifugation step as in **6.2.4.**

6.2.6 Repeat **6.2.5.**

6.2.7 Discard the lower effluent reservoir and invert the upper reservoir onto the retentate cup provided with the microconcentrator unit. Centrifuge at ~500-2000 rpm for 5 minutes to transfer the DNA concentrate into the cup. The final volume of retentate should be ~30-50µℓ.

6.2.8 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

7.0 DNA SAMPLE CLEAN-UP:

DNA samples that have already been extracted may be additionally treated to remove contaminants/inhibitors as needed.

7.1 MICROCONCENTRATOR:

7.1.1 Assemble Microcon-100 according to manufacturers instructions.

7.1.2 Prewet the filter with 50µℓ TE.

7.1.3 Pipette the sample into the upper reservoir.

7.1.4 Fill remaining volume with TE.

7.1.5 Spin in a fixed rotor centrifuge at 2500 rpm for 20 minutes.

7.1.6 Discard the effluent in the lower reservoir as needed.

7.1.7 Repeat steps **7.1.4-7.1.6** two more times.

7.1.8 Remove the filter unit and invert it into a new tube. **OPTIONAL:** 10µℓ of PCR TE can be added to the filter and vortexed briefly before inverting the filter unit into a new tube.

7.1.9 Spin in a fixed rotor centrifuge at 500-2000 rpm for 2-5 minutes to collect sample. The final volume of retentate should be about 30-50µℓ.

7.1.10 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202, BI-204).

7.2 CHELEX CLEAN-UP:

7.2.1 To the DNA sample(s) (~40-50µℓ), add 200µℓ freshly prepared 5% Chelex.
Option: Add 10µℓ Pro K.

Note: This procedure may also be used directly on stains. In this instance, however, 7.2.1 should be preceded by a 15-30 minute RT incubation in 1mℓ of sterile deionized water. Remove all but ~50µℓ of the water before proceeding.

7.2.2 Incubate at 56°C for ≥30 minutes (up to 2 hours).

7.2.3 Incubate in boiling water for 8 minutes.

7.2.4 Spin in a microcentrifuge at high speed (≥ 10,000 rpm) for 3-5 minutes. This extract may be used, however, Chelex extracts perform better when removed from Chelex resin and clean up as follows.

7.2.5 Transfer the solution to a prewetted microcon-100 (50µℓ TE). Care must be taken to not disturb the Chelex resin.

7.2.6 Fill remaining volume with TE.

7.2.7 Spin in a fixed rotor centrifuge at ~2500 rpm for 20 minutes.

7.2.8 Remove the filter unit and invert it into a new tube. **OPTIONAL:** 10µℓ of TE can be added to the filter and vortexed briefly before inverting the filter unit into a new tube.

7.2.9 Spin in a fixed rotor centrifuge at ~500-2000rpm for 2-5 minutes to collect sample. The final volume of retentate should be about 30-50µℓ.

7.2.10 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

8.0 REMOVING MATERIAL FROM SLIDES:

8.1 FREEZING:

8.1.1 Place slide in -80°C freezer for 3-5 minutes.

8.1.2 Wearing safety glasses, pry the cover slip off.

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

8.1.4 Remove the hair and soak in 10-20mℓs 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 differential extraction buffer (see **5.2.1**) 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 **5.2.1.**

8.1.5 Rinse the hair briefly in absolute ethanol to remove the xylene and proceed to hair extraction under **5.3.**

8.2 SOAKING IN XYLENE:

8.2.1 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.

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

8.2.3 Rinse the hair briefly in absolute ethanol to remove xylene and proceed to hair extraction under **5.3.**

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 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). Additionally, using microconcentrator devices for DNA isolation may be more effective in removing heme and other inhibitors than ethanol precipitation.

9.4 These extraction methods employ the use of liquid nitrogen and dry ice. Both of these substances may cause severe burns. Double-glove or wear "cold" gloves while performing these procedures and exercise caution.

9.5 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-208	
8/28/01	9/10/01

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:

ADJ 10% BLEACH or DISPATCH

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.

ADD Conc. of samples 1.1ng preferably (re-extract)

4.1.2 The amount of DNA template added to an amplification reaction should be targeted at 0.5-1.0ng for a single source sample and ~1.0ng-1.3ng for suspected mixtures. For the positive control (9947A), 4ng 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 Amplification reaction set-up is to be performed in the designated biological hood using those dedicated pipets and tips.

4.2.2 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 microAmp tray/rack. The scientist may choose to turn on the UV light at this point (≥ 15 minutes) while performing other preparations.

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

4.2.4 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 samples (primarily offender samples) there is no volume for the DNA template so 19.2µℓ of dH2O will be added to these tubes.

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

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

4.2.7 Pipet 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). 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.

4.2.8 Use the tube-cap sealer to ensure all of the samples tubes are closed tightly. Mix by finger or standard vortex and spin in microfuge to bring the reaction components to the bottom of the tube and remove any bubbles, if necessary. Return samples to MicroAmp tray/rack placing in position for thermalcycler (record position on Form 210-BI).

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

4.2.10 Place the microAmp tray, with the samples, into the thermalcycler. Do not set the microAmp rack down in this room. Remove gloves and return the microAmp rack to biological hood and turn on UV for ~30 minutes.

4.3 THERMALCYCLING PARAMETERS:

4.3.1 After the samples have been placed in the thermalcycler, 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:
59°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:
59°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **20 cycles**, then

60°C for 30 minutes, then:

4°C soak

4.3.1.2 For nonquantified DNA 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:
59°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:
59°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **17 cycles**, then

60°C for 30 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:
59°C for 30 seconds
ramp 23% to:
70°C for 45 seconds
for **3 cycles**, then

60°C for 30 minutes, then:

4°C soak

If, from the data generated on the 310 Genetic Analyzer, it is determined that the signal for a sample falls below 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 should be run through the same process. The positive control will have an excessive amount of product when taken through this process but the scientist may choose to perform the additional cycling and run a dilution on the 310 Genetic Analyzer. If additional DNA extract or sample exists, the scientist should repeat the analysis from that point in addition to, or instead of, performing additional amplification cycles. Cycling additions beyond the 3 cycles provided for in this program would require additional validation and approval of the Biology Program Manager.

Δ Clarify + Ref BI210

4.4 AMPLIFIED DNA PRODUCT:

4.4.1 After cycling has concluded remove samples from thermalcycler. Samples should be run on the 310 Genetic Analyzer as soon as possible after amplification. Prior to 310 run and/or before analysis is completed, the samples may be stored at 4°C for ≤1 week. For longer storage, samples should be frozen at -20°C. Amplified product is **ONLY** stored in the Amp/PostAmp room.

4.4.2 Once 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 sealed in a biohazard container, transported directly to the autoclave, sterilized and disposed of with other biohazardous material.

5.0 COMMENTS: ADD 5.1 BLEACH and UV or paper

5.2 **5.1** Wear gloves at all times during amplification set-up.

5.3 **5.2** Mix all reagents thoroughly (e.g., vortex) and pulse-spin them in microfuge prior to dispensing.

5.4 **5.4** 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.

5.5 **5.5** If DNA template is in TE Buffer, it is recommended that the volume added to the amplification reaction not exceed 20% of the total reaction volume (5µℓ).

BI-210	
8/28/01	

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, 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 Genetic Analyzer with data collection and analysis software employed in developing the genetic profiles, will be used in an effort to individualize biological depositions on evidentiary material and to produce STR profiles on samples from convicted offenders 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

Genescan® Analysis Software User's Manual

Genotyper® Software User's Manual

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 Genetic Analyzer
ABI PRISM™, Genescan® and Genotyper® Software
MacIntosh Computers.
Heating Block (or 480 Thermalcycler)
Benchtop Cooler
Capillaries
Syringe
Sample Tubes and Septa
POP4 Polymer
Genetic Analyzer Buffer
PowerPlex™ 16 Kit Contents
PP16 GenePrint® Matrix Standards
Deionized Formamide

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 Fluorecsein Matrix, JOE Matrix, TMR Matrix and CXR Matrix for the blue, green, yellow and red matrix standards respectively. This is done on each instrument and is performed semiannually, when necessary due to performance, or after any instrument maintenance/repair that involves 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 5 or more days. Fill-in appropriate information in the 310 Injection Log (Form 422-QC).

4.1.2 The ABI PRISM® 310 Collection Software should open upon start-up. 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, put 'CCD DUMMY' and 'SEQFILL DUMMY' as the last two samples. If the sample sheet is full, there is a 'dummy' sample sheet on the instrument so that these samples may be placed in a pre-run by themselves if necessary. 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, NEG, BRB (blood reagent blank), RB (FTA reagent blank), MRB (male reagent blank) FRB (female reagent blank)

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

X= Specimen Type (Q=Questioned; V=Victim; S=Suspect; E=Elimination)
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).
*Paternity-type cases will be preceded by the letter 'G' and sample types as follows: M=Mother; F=Alleged Father; C=Child; FB=Paternal uncle; FS=Paternal Aunt; FM=Paternal Grandmother; FF=Paternal Grandfather, etc.

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 every 20-25 samples in a long run. Move the 'CCD DUMMY' and 'SEQFILL DUMMY' to the 1st and 2nd injection positions, respectively. 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: 5secs for Matrix Standards
3secs for Allelic Ladders and 1ng POS control DNA (injection times may be adjusted [3-10 seconds] but a 3 sec. inj. time for single-source samples estimated at ≥ 1ng and 5 sec. for 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 ~0.33µℓ ILS600 size standard/sample; 24µℓ deionized formamide/sample and adding quantities for N+2 in Master) that has been dispensed into sample tubes. For Allelic Ladders add ~0.25µℓ-1µℓ Ladder to 25µℓ Master Mix. Option: A Ladder Master Mix may be made up using these proportions and stored frozen in 25µℓ aliquots.

Matrix samples are added to 25µℓ of deionized formamide (without size standard). Note: due to the fact that the 'virtual filters' in this instrumentation are not optimal for the dyes used in this kit, the amount of each matrix standard to be run for optimal color subtraction may vary and will be determined empirically for a matrix run. However, a starting point of 25µℓ of a 1:25-1:40 dilution (in formamide) of each Matrix standard will generally produce an acceptable matrix. See Promega Matrix product bulletin for spectral overlap and matrix correction.

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 ≥5 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 (APPLE - I) and select that sample from the pull-down menu, changing the injection time if necessary.

4.1.11 After completion of run, finish filling out 310 Injection Log (Form 422-QC), print final Genescan® Injection List (65%) and place in QC binder.

4.2 DATA ANALYSIS: GENESCAN®

4.2.1 Data analysis (with the exception of Matrix generation) is NOT performed on the instrument computers (Mendel or Ros). Make a copy of the Run Folder and, using chooser file-sharing, transfer the copy to your 'Runs to Analyze' folder on an analysis computer. Make another copy of the Run Folder and transfer it to the 'Original Run Files to be Archived' folder on Fleetwood's Desktop. The runs in this folder will be archived to CD. Once this has been performed, the Run Folder on the instrument computer (Mendel or Ros) will be deleted.

4.2.2 Create Genescan® Project:

For Matrix generation and CODIS Runs, open the project that should have been created automatically or open Genescan® and use FILE\NEW\GENESCAN PROJECT\ to bring in the appropriate samples.

For individual cases, make a copy of the Run Folder for each case and change name of folder to the laboratory case number, as they will be separate projects. Open Genescan® and use FILE\NEW\GENESCAN PROJECT\ to bring in the appropriate case samples, controls and ladder(s). Delete samples from other cases from the new 'Case Run Folder'. When project analysis is complete save project as the lab case number.

4.2.3 Set the parameters for Genescan® Analysis:

For Matrix Generation, review the Raw Data of the Fluor, JOE, TMR and CXR standards 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.

Use FILE\NEW\MATRIX to bring up a window to select the analysis parameters. Click on a 'dye color' and select the corresponding Matrix Standard (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 'OK' and a new Matrix file will be generated.

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). Use FILE\SAVE AS\ to save new Matrix file (name is letter for instrument [M or R] followed by "MATRIX" and then the date "MMDDYYYY").

Check Matrix quality by applying it to the matrix samples. Select those samples in the Analysis Control Window and, using Project\Install\New Matrix, apply Matrix and analyze the data. Examine data in the Results Control Window. 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, save a copy of the Matrix to the ABI Folder in the System Folder (make color correction matrices if necessary). Print out Matrix Table and a 4-color electropherogram plot, from the Results Control Window, for each Matrix Standard. File in QC log for appropriate instrument.

For standard runs, review the raw data for all of the project samples and controls to determine the start and stop points of the analysis range. Select points that will not include the primer peaks but will cover the size range of 80 to ≥500 bases. Standard settings are as follows:

Analysis Range: This Range (empirically determined for each run but typically ~3400 start to 8100 stop)

Data Processing: Baseline and Multicomponent with light smoothing.

Peak Detection: Generally 150 rfu in all colors. Rfus may be raised in Blue, Green and Yellow for Allelic Ladder or CODIS samples only. Rfus may be lowered if necessary to ≥ 75 rfu (≥50 rfu requires Technical Leader approval; <50 inconclusive).

Size Call Range: This Range; Min=80, Max=600

Size Calling Method: Local Southern

Split Peak Correction: None

To avoid building up a huge GS Parameter File, use 'SAVE', instead of 'SAVE AS' and just save the settings as the default <Analysis Parameters>. A scientist will have to save parameters under another name in the event a run file requires more than one set of parameters. (e.g. LDC, 200 RFU)

Size Standard ILS600 with defined peaks at 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, and 600 bp.

To avoid building up a huge GS StandardS File, use 'SAVE', instead of 'SAVE AS' to save the defined size standard as <ILS600>.

4.2.4 GENESCAN® DATA EVALUATION

4.2.4.1 Review Raw Data in the Analysis Control window 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.

4.2.4.2 After applying a matrix, defining Analysis Parameters and Size Standard, highlight the samples (gray square in upper left corner will select all samples and colors for analysis) to be analyzed. Click Analyze.

4.2.4.3 In the Results Control Window, the scientist may examine all colors of a given sample simultaneously to identify bleed-through, spikes, etc. By simultaneously viewing the data table, it is easy to discern possible stutter, -A peaks etc. The scientist may verify the correct assignment of size standard peaks or do so in Genotyper®.

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

4.2.4.5 Review all samples and evaluate: peak height and shape, matrix quality, and individual sample profiles. It is a good time to determine if the rfu cut-off will need to be changed in the Analysis Parameters of certain samples. Rfus should ideally be < 4000. However, data may be acceptable to 6,500 rfus for forensic data and higher for CODIS offender samples.

4.2.4.6 After analysis and review are complete, SAVE PROJECT AS: Laboratory Case Number or CODIS RUN"MMDDYY" or other appropriate name for QC, validation or research project.

4.2.4.7 For case file documentation, Genescan® plots and data tables are only printed for documentation of anomalies within an allelic range that could interfere with either computer analysis or scientist's analysis. For example, for a spike that exceeds 150 rfu and falls within the allelic range of a given locus, the scientist would print the 4-color plot with data table, demonstrating the spikes presence in all 4 colors at the same (approximate) size. The spike would also be marked on the Genotyper® Plots -that will be printed for the case file. Once the case analysis is completed and has been reviewed (i.e., Genotyper®) the case folder will be burned to a scientist's 'cases' CD; a case-specific CD will be made for discovery when requested.

4.3 ALLELE ASSIGNMENT: GENOTYPER®

The PowerTyper™ 16 Macro is used in conjunction with Genotyper® software to automatically convert allele sizes imported from Genescan®, to allele designations. Genotypes are assigned by comparing the sizings of unknown alleles from samples with the sizings of known alleles contained within the allelic ladder of each locus.

4.3.1 Open the PowerTyper™ 16 Macro and import a Genescan® project or sample files (all 4 colors).

4.3.2 Double-click on the 'Check ILS' macro. Examine the size standard of each sample to confirm correct assignment of fragment sizes. If necessary, re-analyze in Genescan® and re-define the size standard.

Note: if additional peaks are assigned because of bleed-through of TMR peaks, scientist may go back to Genescan® and change the Analysis Parameters, increasing the rfu threshold (or use CC matrix) for the red channel to prevent these peaks from being detected.

4.3.3 Double-click on the 'Power' macro that will take a few minutes to identify alleles in the ladder sample and calculate offsets for all of the loci.

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

Note: Only one ladder sample is used for determining allele designations and the macro automatically uses the first ladder sample. If anomalies such as many off ladder alleles appearing in the samples, the scientist should use another ladder and re-run the Genotyper® analysis.

4.3.5 Double-Click on the 'Display Fluorescein Data' macro to display the blue dye for all samples. Scroll through each sample, comparing it with the allelic ladder and examine for off-ladder variants, 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., and edit as necessary.

4.3.6 Run the 'Display JOE Data' and 'Display TMR Data' to similarly examine the green and yellow dye plots, respectively.

4.3.7 Create an allele table by running one of the 'Make Table' macros. The only table that will accept more than 2 alleles per locus is the PowerTable. If none is suitable, you can use TABLE\SET-UP to create one appropriate for the data. Use TABLE\EXPORT TO FILE to save the table (same name as project and add "Allelic Table") and make it available for editing in Excel.

4.3.8 Print (at 75%) the Genotyper® Plots for case files. Review of CODIS data will be performed on a computer and a copy of the electronic data will be burned to CDs for archiving. The Genotyper® plots and CODIS Allelic Table will be printed and placed in a CODIS Data folder.

4.3.9 Before exiting Genotyper®, perform a **SAVE AS!!!- or you will overwrite the PowerTyper™ 16 Macro!!** For case projects, title "Case Number" Genotypes, for CODIS projects, "CODIS RUN MMDDYY" Genotypes.

4.3.10 Samples demonstrating an off- ladder (those either smaller or larger than the smallest or largest ladder allele, respectively) or microvariant (alleles of sizes indicating incomplete repeats) allele(s) should be re-analyzed for verification where necessary (evidentiary profile in nonsuspect case). Micro variants will be 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.

4.4 STR INTERPRETATION GUIDELINES AND STATISTICAL ANALYSES

4.4.1 CONTROLS

4.4.1.1 The purpose of a **REAGENT BLANK** is to determine if the reagents used to extract the samples were contaminated by human DNA. In the Genescan® 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, fluorscent '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. Data may be acceptable if contamination is 'isolated' to the RB or insignificant relative to sample signal.

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. If the expected alleles (see below) are not detected, results for all of the samples associated with amplification are deemed inconclusive.

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	AMLGNN	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 human DNA contamination occurred in the process of amplification set-up (or somewhere from that point on). In the Genescan® 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 should 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 are made in batches and given random numbers. The scientist is not aware of the genotype of the sample. A blind control will be run with every extraction set. The reviewing scientist will complete a Blind Control Check Form (Form 212-BI) for verifying correct genotype. 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 extraction or pre-extracted blind control DNA may be used.

4.4.2 RFU THRESHOLD:

4.4.2.1 For single-source forensic samples (sufficient in quantity and condition) and reference standards (excluding autopsy samples that may be degraded or of limited quantity), a minimum of 100 rfu must be achieved. 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 PCR added for fragment analysis), or re-amplification, or re-extraction.

4.4.2.2 For the detection of mixture components (or low-copy single-source forensic samples), a threshold of 50 rfus may be used if warranted by the scientist (and approved by the technical leader). However, extreme caution should be used in the interpretation of mixture components in the 50-75 rfu range and, depending on other factors (e.g., baseline and background signal), the scientist may make the determination that they are inconclusive for reporting.

4.4.2.3 Peaks below 50 rfu are inconclusive for inclusionary purposes but may, at the discretion of the scientist (based upon their knowledge and expertise in conjunction with the data obtained), be reported for exclusionary purposes only.

4.4.2.4 Peaks >8000 rfus will not be interpreted; the sample must be diluted, re-injected, reanalyzed or re-amplified as deemed appropriate by scientist. Peaks between 4000-8000 rfu may be interpreted or repeated at the scientist's discretion. Application of a CC matrix may be necessary for higher rfu samples.

4.4.3 MIXTURES

4.4.3.1 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.

4.4.3.2 Loci that possess only two alleles at a locus 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 at additional loci.

4.4.3.3 Mixture assessment, in terms of determining the presence of a mixture and probable locus genotypes is performed prior to examining the reference profiles.

4.4.3.4 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 was predominant (i.e., the "highest rfu peak" in the mixture may not be the shared allele).

4.4.3.5 For loci where distinct genotypes are discernible (this will occur rarely in a mix of more than two individuals' DNA), both genotypes may be reported.

4.4.3.6 A sample with interpretable peaks at one or more loci may be reported even if no peaks are detected at additional loci; statistical interpretation will demonstrate the significance (or lack thereof) of the data.

4.4.4 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 bases of all calculations used in the reporting of forensic "matches".

4.4.4.1 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 DNA View programs. Additional population data may also be used when available and relevant to a particular case (See BI-400 for reporting of statistical frequencies).

4.4.4.2 The frequency for a heterozygous profile is determined by the equation $f_{(pq)} = 2pq$

4.4.4.3 The frequency for a homozygous profile is determined by the equation $f_{(pp)} = p2+p(1-p)\theta$, where $\theta = 0.01$ except where small isolated populations (e.g., Native Americans) may be relevant, in which case, $\theta=0.03$.

4.4.4.4 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 $f_{(STR\ profile)} = 2 \times 10^{-14}$, RMP= 1 in 5×10^{13}; See BI-400) for reporting of statistical frequencies).

4.4.4.5 For mixtures for which distinct genotypes are not discernible, 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 BI-400) 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 $P_{Ecombined}$ (for all of the loci combined) is as follows:

$P_{Ecombined} = 1 - [(1- P_{E1})(1- P_{E2})(1- P_{E3})\ldots(1- P_{E15})]$
(See BI-400) for reporting of statistical frequencies).

4.4.4.6
In addition to the LR and PE used in paternity, the probability of paternity may be used. However, given that this statistic requires nongenetic information (i.e., the prior odds of paternity), the prior odds used (e.g., 50%) should be explicitly stated (See BI-400 for reporting of statistical frequencies).

4.4.4.7
In many forensic cases, the inverse 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., α=0.01; 1-α=99% confidence).

The FBI has selected an upper confidence limit (UCL) of 99% (α=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 $>3.9 \times 10^{-11}$ (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 BI-400) for reporting of statistical frequencies).

BI-301	
8/28/01	9/04/01

CODIS SAMPLE RECEIPT AND STiMAS 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.

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:

CODIS Computer Workstation
Barcode Equipment
Database Samples and Report Forms

4.0 PROCEDURE:

REVISED TO D IN PROTOCOL

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 are mailed to the laboratory separately; in the event that either one has not been received, the submitting agency should be notified.

4.1.2 Compare the DNA sample card information to that of the Collection Report Form and verify that it is the same. Search the 'to be collected' offender database and, if the offender is in that database, indicate receipt of sample and collection report by checking the appropriate 'received' box.

4.1.3 Make a copy of the sample card information and fingerprint sections to be forwarded for fingerprint verification.

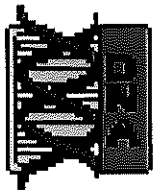
4.2 STiMAS ENTRY:

4.2.1 Log on to the CODIS workstation computer and open the STiMAS database program. Open the 'Convicted Offender' application and select 'Submissions' from the menu.

4.2.2 Enter the sample card and report form information into the appropriate fields. Choose save for assignment of a unique barcode number and print the corresponding labels by clicking on the 'Barcode Labels' button.

4.2.3 Place a barcode label on the DNA sample card and another on the Collection Report Form. A third label is attached to the FTA card envelope to be placed on the card at the time of DNA testing. An additional label may be printed and attached to the FTA envelope for use with a barcode scanner during STR Sample Sheet data entry.

4.2.4 Place the DNA sample card in a locked filing cabinet located in the laboratory for storage and file the report form in the database cabinet.



Form 204-BI

QUANTIBLOT WORKSHEET

Scientist _____ **ImageID** _____

Date: _____

Quantiblot Kit Lot# _____ **Spotting Solution Lot# QSS** _____

Membrane Source _____ **Lot#** _____

Hybridization Solution Lot# QHB _____ **30%H_2O_2 Lot#** _____

Wash Buffer Lot# QWS _____ **Citrate Buffer Lot# QCB** _____

Chemiluminescence Reagents Lot# _____

Record sample ID and ng detected in table below. Attach Image Station data. Placement of standards may be varied by scientist.
Note: Image station defines whole column as 'standard' so no samples may be placed in a column with standards.

A1 STD 10NG	A2	A3	A4	A5	A6
B1 STD 5NG	B2	B3	B4	B5	B6
C1 STD 2.5NG	C2	C3	C4	C5	C6
D1 STD 1.25NG	D2	D3	D4	D5	D6
E1 STD 0.625NG	E2	E3	E4	E5	E6
F1 STD 0.313NG	F2	F3	F4	F5	F6
G1 STD 0.156NG	G2	G3	G4	G5	G6
H1 STD ØNG	H2	H3	H4	H5	H6

REVISED

FORENSIC BIOLOGY WEEKLY QC

Form 404-QC

DATE												
	°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												

DATE				
NANOPURE SYSTEM /20				
EYE WASH STATION				
CHANGE TEMP CHART				
°C WATER BATH A (BCIP)				
°C WATER BATH B (SHAKER)				
°C WATER BATH C (REAGENTS)				
°C OVEN				
°C HEATING BLOCK				

*Performed by Biology/CODIS Lab Tech; other personnel as arranged by Tech.

FORENSIC BIOLOGY CHEMICAL INVENTORY

Form 400-QC

Chemical/Source	Lot Number	Exp. Date	Date Rcvd	Qty Rcvd	Qty In Stock

New Electronic - Based Form

FORENSIC BIOLOGY QUARTERLY QC

Form 408B-QC

SCIENTIST:_____ **QC DATE:**_____

IV. Chemical Safety Shower Check_____

V. Forensic Biology Reagent Balance Check

Nist-traceable 1.0g wt. Observed wt._____ Pass☐ Fail☐

Nist-traceable 0.1g wt. Observed wt._____ Pass☐ Fail☐

Observed weights should fall between ±0.03g actual weight. If the balance fails it should be serviced.

ADD CENTRIFUGE

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.

Run Date: _____ Run Folder: _____

QA/QC PASSED: YES ☐ NO ☐

Comments:

Attach the appropriate extraction/amplification/BC forms used and the Genotyper Electropherograms; place in Forensic Biology QC Binder.

NOTE ON CODIS AS QC

INSTRUMENT: MENDEL ROS

Form 422-QC

310 INJECTION LOG

DATE	ANALYST	FORMAMIDE CHANGED √ (LOT#)	H_2O CHANGED √	BUFFER CHANGED √ (LOT#)	POP-4 CHANGED √ (LOT#)	#INJECTIONS RUN/TOTAL	CAPILLARY CHANGED √ (LOT#)	CASES/SAMPLES RUN	RUN FOLDER

4 more space if row

Form 424-QC

MONTHLY 310 QC RUN

SCIENTIST:_____ **QC DATE:**_____

Once a month, a positive control and ladder sample will be run on both instruments. Each sample will be injected ten times consecutively. the following data will be gathered from these injections. The rfu and bp size values for all of the alleles in the 9947A DNA samples and the following ladder *alleles will be collected and analyzed: D3S1358-16, Penta E-5, D5S818-14, Penta D-13, FGA-24.4, and FGA-20.

*These ladder alleles typically represent the lowest and highest rfu values in blue, green, and yellow, respectively.

An Excel Spreadsheet will document all the values (size and rfu) and calculate the MIN, MAX, MEAN and STD DEV.

This spreadsheet will be placed in the instrument QC binders. Once a year (or if noticeable change occurs), the monthly data will be compared to one another and graphed to follow trends.

The Genescan® and Genotyper® Data will be analyzed as usual and quality of results will be reflected in the comments or 'passed' areas; no hard copy of the data will be printed. Data will be burned to QC CD.

Run Folder: _____

QC PASSED: YES ☐ **NO**☐

Comments:

Also to Quarterly QC

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. A minimum of two Blind Control samples will be analyzed simultaneously.

NO BC REQUIREMENT

The Genescan® and Genotyper® Data will be analyzed as usual and quality of results will be reflected in the comments or 'passed' areas. The Genotyper® 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:

8.0 CALIBRATION AND MAINTENANCE

General laboratory procedures for the calibration and maintenance of equipment is covered in the ISP Forensic Services Quality Manual. See MBI-400(4.2), in Section 7 of this manual for practices specific to Forensic Biology.

9.0 PROFICIENCY TESTING

General laboratory guidelines and practices for proficiency testing are outlined in the ISP Forensic Services Quality Manual. Additionally, for Forensic Biology, DNA analysts will participate in external proficiency tests, twice in every calendar year with an interval not to exceed 183 days between tests. Proficiency testing for these personnel will be reported to NDIS as necessary.

ADDRESS PT Δs (NDIS)
INC/UNINTERPRETABLES

10.0 CORRECTIVE ACTION

Laboratory corrective-action procedures are detailed in the ISP Forensic Services Quality Manual.

11.0 REPORTS

Policies and practices on reports issued in Forensic Biology are detailed in MBI-400(4.3.2) in Section 7 of this manual.

12.0 REVIEW

Laboratory 'casework review' is addressed in the ISP Forensic Services Procedure Manual. Review of documentation in Forensic Biology is delineated in MBI-400 (4.3.3) in Section 7 of this manual. See also, forms 214-BI and 306-BI in Section 7 of this manual. 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 Program Manager 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 Safety Manual. In Forensic Biology, personnel are introduced to these practices in Module 1 of the ISP Forensic Biology Training Manual. In addition, forms 404-QC and 408B-QC (Section 7 of this manual) address the monitoring of safety devices, the chemical eye wash and shower, respectively.

14.0 AUDITS

Quality audits are delineated in the ISP Forensic Services Quality Manual. That section addresses the additional requirement for DNA that in alternating years, the audit must be performed by an external auditor.

ADDRESS NEW NDIS REQUIREMENTS