ISP FORENSIC BIOLOGY QA MANUAL

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APPROVED BY: ______________________
Biology/DNA Supervisor   Date: 2/4/2003

____
Lab Improvement Manager   Date: 2-4-03

BI-QA
Revision 1
02/2003

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.

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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 5.2 and 5.3 of these documents, respectively). A copy of each may be found in the ISP Forensic Biology Training Manual.

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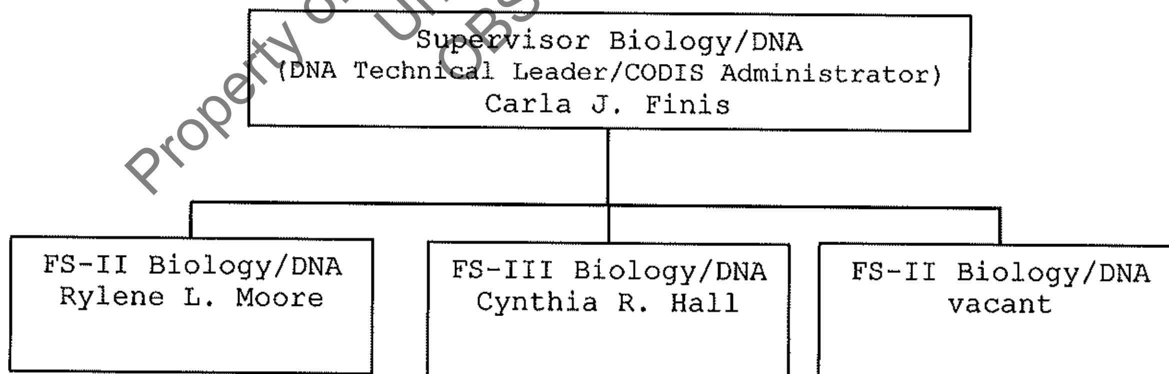

Note: Changes (personnel) to this page do not require new revision number.

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/DNA Supervisor/Technical Manager

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

3.3.1.1 Education

Must have 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.

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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/DNA Supervisor.

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.

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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.

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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.

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CARLA J. FINIS, Ph.D.

TITLE: Biology/DNA Supervisor

EDUCATION: B.S., Genetics
University of Minnesota

Ph.D., Genetics
University of Minnesota

CERTIFICATION: American Board of Criminalistics Diplomate; Fellow in Molecular Biology

EXPERIENCE: 1999 - present: Idaho State Police
Forensic Services

1990-1999: MN Bureau of Criminal Apprehension
Forensic Science Laboratory

ADDITIONAL TRAINING:

In-service training: Serology (BCA)

In-service training: DNA/RFLP (BCA)

Forensic Applications of DNA Typing Methods - FBI (Quantico) through the U of VA

Advanced Aspects of Forensic DNA Typing - FBI

In-service training: Courtroom Testimony - BCA

In-service training: Crime Scene Processing - BCA

Bloodspatter Workshop - MAFS

Workshop: Statistical and Genetic Considerations for Forensic Analysis - MAFS

In-service training: DNA/PCR-DQA1, PM and D1S80 - BCA

In-service training: STRs - ABI 377/310 - PE Biosystems at BCA

In-service Training: STRs in Forensic Casework/Troubleshooting and Interpretation - RCMP at BCA

In-service Training: STRs and related Statistics - Dr. George Carmody/Carleton College at BCA

Professional Organizations: American Academy of Forensic Sciences

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CYNTHIA R. HALL, M.S.

TITLE: Principal Criminalist-Biology/DNA

EDUCATION: B.S., Biochemistry
University of California; San Diego

M.S., Molecular Genetics
San Jose State University

EXPERIENCE:

2001 - present: Idaho State Police
Forensic Services

1995-2001: Santa Clara County DA's Office
Crime Laboratory

1994-1995 California Department of Justice
DNA Laboratory

ADDITIONAL TRAINING:

In-service training: Serology (SDPD, SCCCL)

In-service training: DNA/RFLP (CA DOJ)

Forensic Serology - CA Criminalistics Institute

Polymerase Chain Reaction - CA Criminalistics Institute

In-service training and Workshop: DNA/PCR-DQα and D1S80 (CA DOJ)

Crime Scene Investigation I - CA Criminalistics Institute

Forensic Statistics on DNA Analysis - CA Criminalistics Institute

Short Tandem Repeat Analysis - CA Criminalistics Institute

Forensic Statistics - CA Criminalistics Institute

CODIS Local training - FBI/SAIC

In-service training: STRs - ABI 377/310 - Applied Biosystems at SCCCL

Crime Scene Reconstruction - Tom Bevel and Ross Garner

Professional Organizations:

American Academy of Forensic Sciences

California Association of Criminalists

Association for Crime Scene Reconstruction

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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 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 is accessible, only to personnel designated by the Biology/DNA Supervisor.

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

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

4.2 Forensic Biology Laboratory Set-up

The Forensic Biology Laboratory is designed to minimize contamination potential during the processing and analysis of forensic and CODIS samples. The diagram below depicts the laboratory set-up and delineates the separate areas for evidence examination, DNA extraction, PCR Amplification Set-up and Amplified DNA processing and storage.

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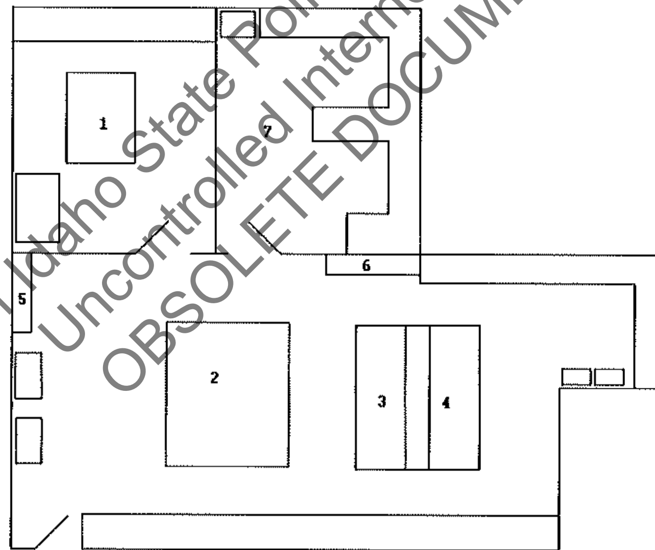

Biology Lab Areas

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 (including CODIS samples) that is collected, received, handled, sampled, analyzed and/or stored by ISP Forensic Services is done so in a manner to preserve its identity, integrity, condition and security.

5.1 Laboratory Evidence Control

Procedures detailing evidence handling are contained in the ISP Forensic Services 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 non-probative casework analyses that are necessary for the validation of any new technology. Therefore, a DNA packet is created for each case (containing reference(s) or a positive Biological screen) 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.

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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.

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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-105 O-TOLIDINE TEST FOR BLOOD
BI-106 HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST
BI-108 SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION
BI-110 BIOLOGICAL SCREENING: USE OF ALTERNATE LIGHT SOURCE
BI-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-302 CODIS SAMPLE DATA ENTRY AND UPLOAD
BI-303 CODIS DATABASE HIT VERIFICATION
BI-310 CODIS SAMPLE EXPUNGEMENT

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

100-BI PHENOLPHTHALEIN REAGENT
101-BI BIOLOGY SCREENING SUMMARY
102-BI HYDROGEN PEROXIDE 3% (v/v)
103-BI O-TOLIDINE REAGENT
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 SOLUTION (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)

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247-BI	CITRATE BUFFER pH5, 0.1M (QCB)
249-BI	BOVINE SERUM ALBUMIN (BSA) 4%
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
406-QC	FORENSIC BIOLOGY MONTHLY QC
408A-QC	FORENSIC BIOLOGY QUARTERLY QC
408B-QC	FORENSIC BIOLOGY QUARTERLY QC
410-QC	QC ABACARD® HEMATRACE® KIT
412-QC	QC ONESTEP ABACARD® P30 KIT
414-QC	PCIAA QC
416-QC	QUANTIBLOT KIT QC
418-QC	ACES KIT QC
420-QC	QC STR KITS
422-QC	310 INJECTION LOG
424-QC	QUARTERLY 310 QC RUN
426-QC	ANNUAL NIST QC RUN

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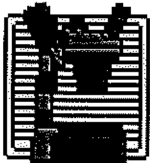

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EXAMINATION OF BLOODSTAINED EVIDENCE

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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:

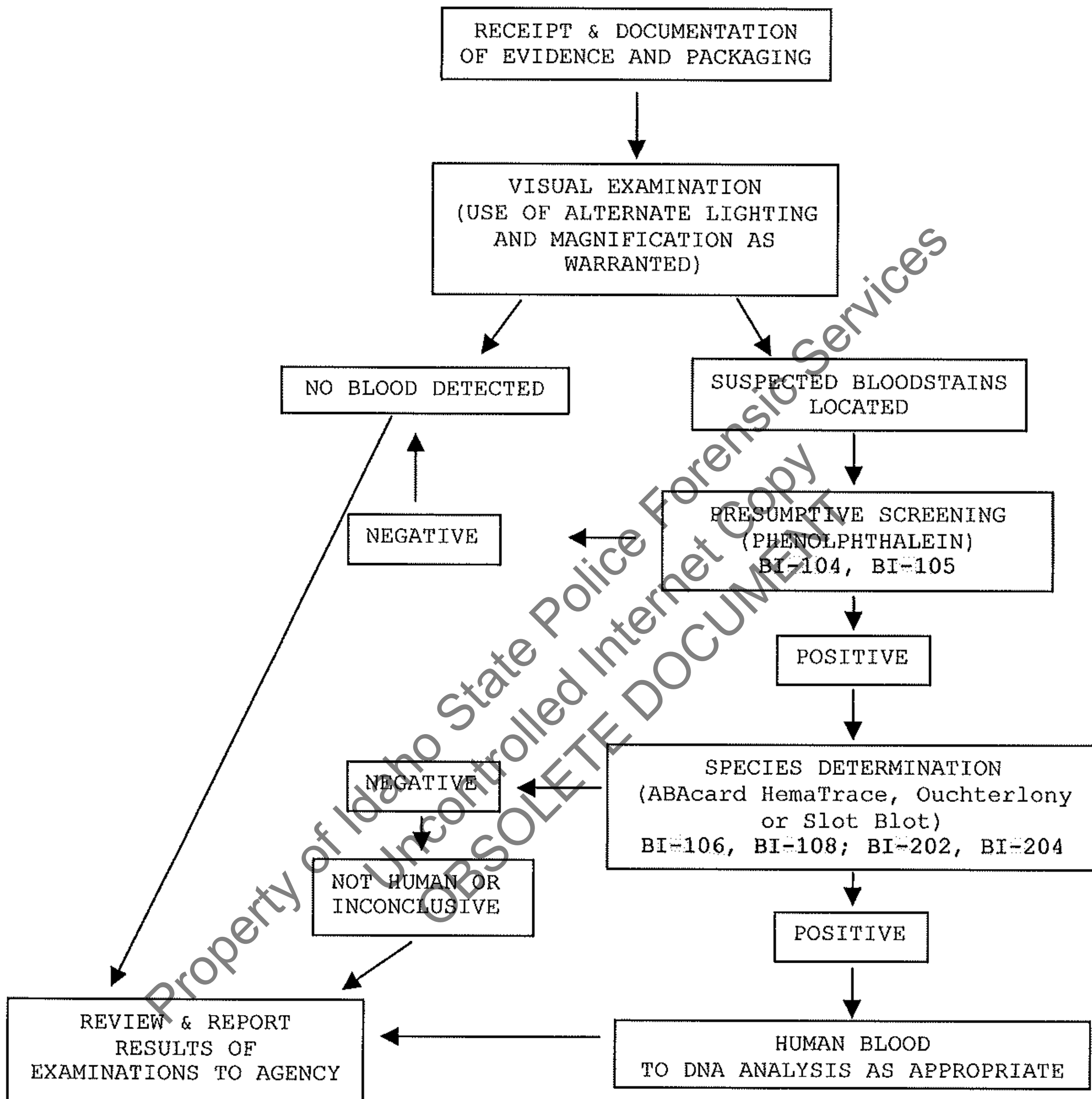
To provide uniform processing of evidentiary material for the presence of blood.

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.



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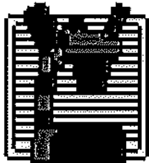
5.0 COMMENTS:

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.



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EXAMINATION OF EVIDENCE FOR BODY FLUIDS

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1.0 BACKGROUND:

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

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

2.0 SCOPE:

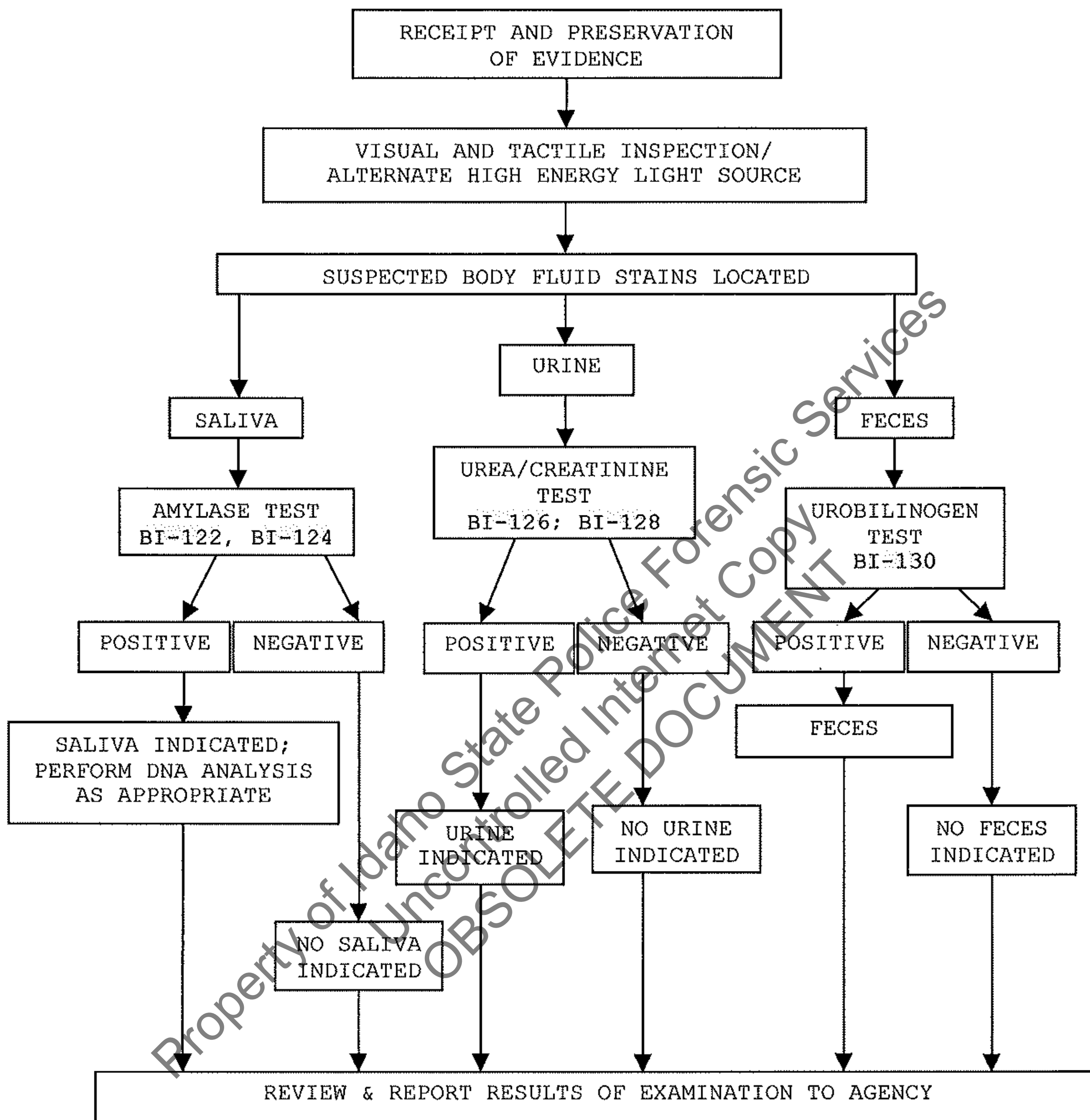
To provide uniform processing of evidentiary material for the presence of saliva, urine or feces.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

See Flow Chart on following page.



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5.0 COMMENTS:

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

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

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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.

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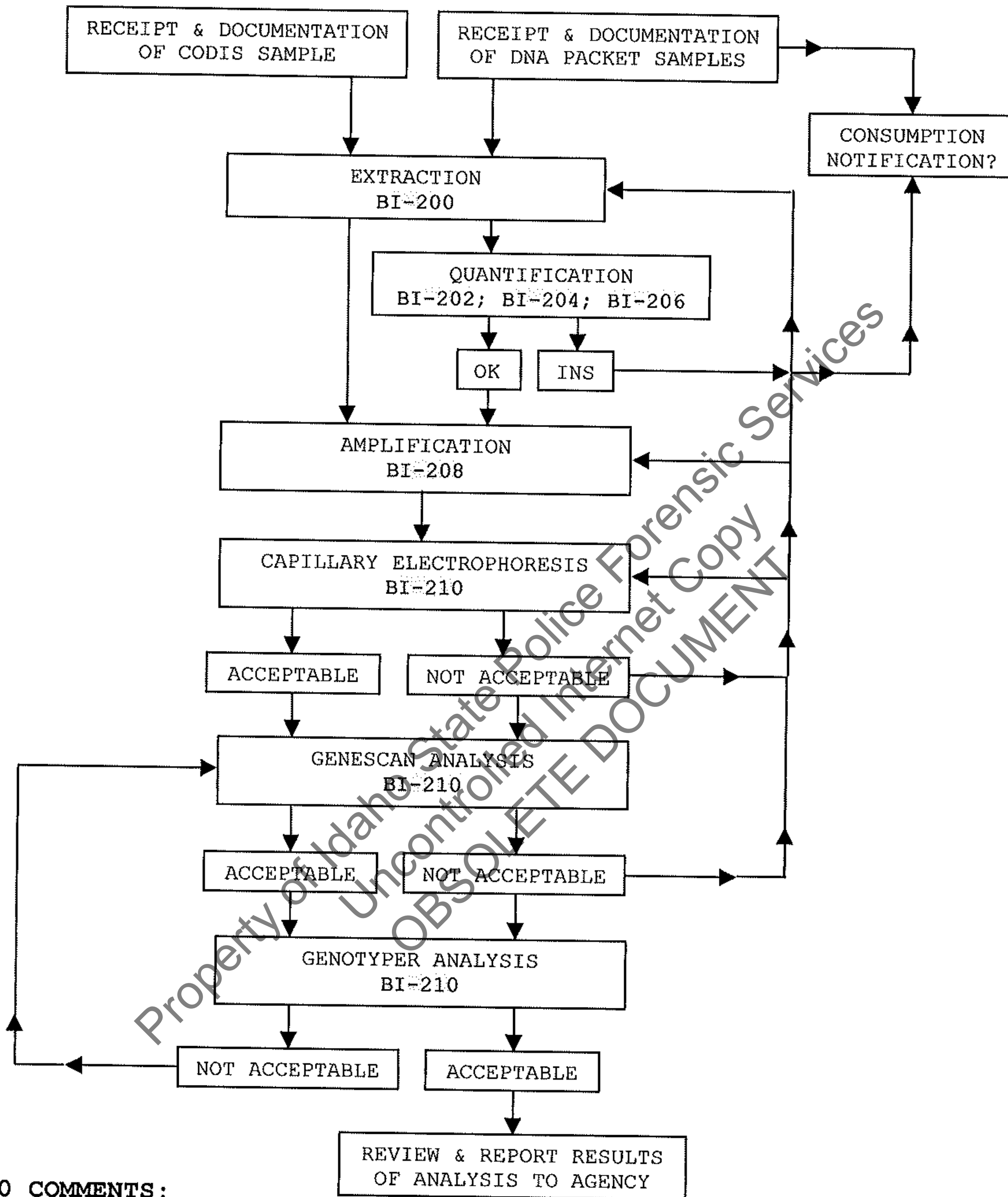

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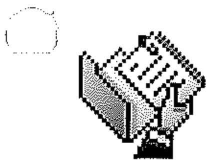
5.0 COMMENTS:

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



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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.

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4.0 PROCEDURE:

4.1 CHEMICALS/REAGENTS

4.1.1 COMMERCIALLY PURCHASED CHEMICALS

4.1.1.1 Biology Personnel should consult the electronic Chemical Inventory Log (Form 400-QC) prior to ordering. The ordering should be reflected in the log to avoid duplicate orders. This inventory will be audited 2X/year (in January and June) and a printout placed in the Forensic Biology Reagent Binder.

4.1.1.2 Upon receipt of a chemical or kit, a new entry will be made in the Chemical Inventory log (note: a new 'Chemical' entry is only necessary when it is an item not in current inventory). The chemical(s) will be marked with the date received and the individual's 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://msds.pdc.cornell.edu/msdssrch.asp 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.

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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 NFPA designation will be completed on all labels (see reagent sheets). Although the reagent is identifiable to lab personnel by lot number (which consists of the first few letters of the reagent name followed by the date in the form 'MMDDYY'), the reagent label should still bear the name of the reagent as well. Refillable squirt-bottles of water or ethanol will be labeled but need not bear dates or initials.

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4.1.3 CRITICAL REAGENTS

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

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 (Taq Polymerase checked with kits; 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 spreadsheet 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 the instrument 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 are reported on Form 402-QC.

4.2.5 The SCHEDULE of QC Checks is as follows:

WEEKLY (Form 404-QC)

Nanopure and RO Water System Check
Refrigerator/Freezer Temperature Check
Eye Wash Station Check
Water Bath(s) Temperature Check
Heating Block Temperature Check
Oven Temperature Check

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MONTHLY (Form 406-QC)

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

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)
Centrifuge RPM Check (Form 408B-QC)
STR QC Run (Form 424-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)
Microscope cleaning/preventative maintenance

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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 a form indicating page/total pages).

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

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

4.3.1.4 The description of evidence packaging should include the type and condition of seal(s). Differences in the description on a package versus ETS entry (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 the original seals. Any altered seal 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).

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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:

$\leq$ 1 area tested positive for a biological substance ≡ Item 57

$\geq$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 ≡ Item 1A-1, Item 1A-2 etc.

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. In general, subfolders should be organized from front to back as follows: report, chronological case notes/forms, copy of chain of custody, phone/info log (on 'tangerine' paper), followed by 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.

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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:

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

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

Signature

____

Name of Scientist
Title of Scientist

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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 (<u>all</u> items not examined will be included; reference samples do not fall into this category):

"Preface see above" were not examined.

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.

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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)].

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".

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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 ≤ 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 probative 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 "X" persons. "Name(s)" is a potential contributor(s) to this mixture. "X%" of unrelated individuals randomly selected from the general population would be expected to be eliminated as potential contributors to this mixture.

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Or: (two person mixture example)

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 statements 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 in any report that has the 'source attribution' statement:

[1]This conclusion is based upon the following: 1) a genetic match at the gender identity locus, Amelogenin, in addition to the following "number" polymorphic STR loci listed below that have an expected population frequency of less than 1 in "actual (most conservative) frequency estimate", 2) a statistical frequency exceeding the source attribution criterion (for $N=1.6\times10^7$, $\alpha=0.01$; *Forensic Science Communications 2(3) July 2000,* and 3) 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.

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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 (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.

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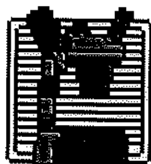

BI-100	
2/4/2003	2-4-03
[signature]	[initials]

PROCESSING LIQUID BLOOD

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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:

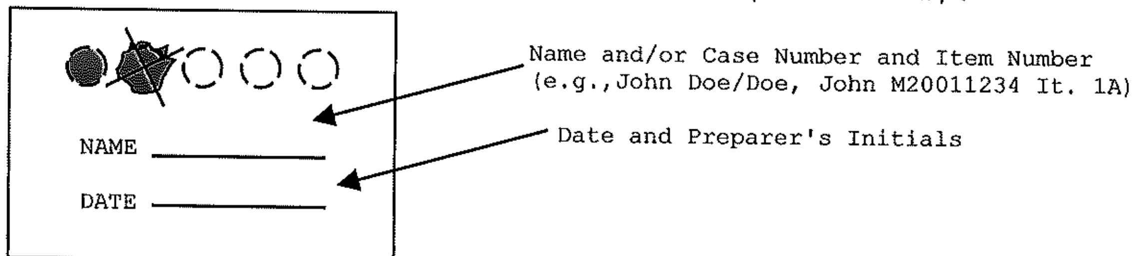
Schleicher & Schuell: Blood Collect Card(s) #903
Sterile, Disposable Transfer pipet
or 1 mℓ pipet with sterile tip

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).



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 <u>COMMENTS</u>:

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.

5.3 The bloodstain card should be labeled with the case number, item number, date and analyst's initials. Typically, the name of the individual is also included.

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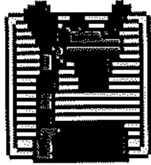

BI-102	
2/4/2003	7/4-03
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DNA PACKETS

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1.0 BACKGROUND:

It has become increasingly important to retain evidence for possible future analyses and to secure samples for nonprobative casework analyses that are necessary for the validation of any new technology. Therefore, where possible, a DNA packet is created for each case that is submitted for analysis to Forensic Biology.

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).

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.

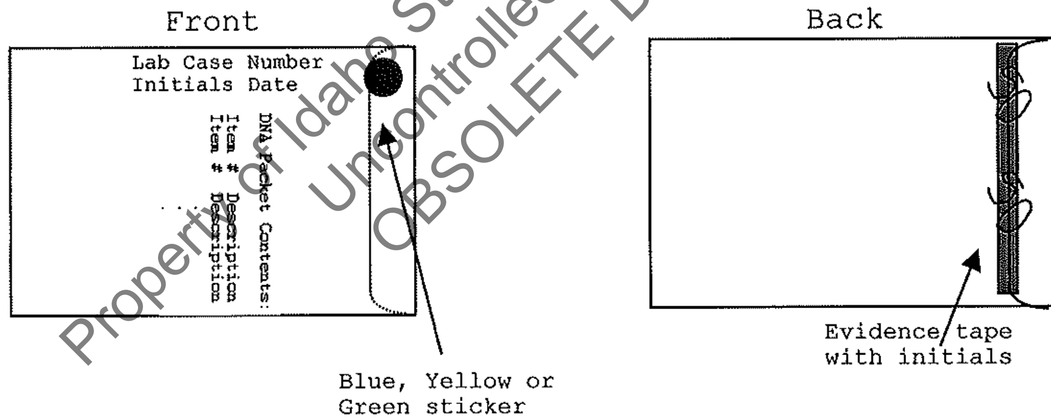
4.2 All sealed coin envelopes will be placed inside a larger manila envelope (DNA Packet Envelope) and labeled as below.

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

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

4.5 Once sealed, the DNA Packet will be taken to a FES and entered as an additional item of evidence ("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 $\leq -20^{\circ}C$ 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/DNA Supervisor should be notified to ensure maintenance on site is no longer necessary.



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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".

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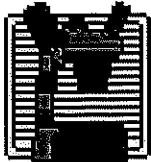

BI-104	
2/4/2003	2-4-03
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PHENOLPHTHALEIN TEST FOR BLOOD

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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 (known 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 ($\leq$ 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.

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BI-105	
2/4/2003	2-11-03
[signature]	[signature]

O-TOLIDINE TEST FOR BLOOD

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1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

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

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

4.4 Add 1-2 drops of 3% H_2O_2 and note appearance or absence of blue-green color. Color reaction should occur rapidly (≤ 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 Direct testing of a small cutting/sample may also be performed.

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

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

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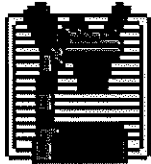

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HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST

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1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABACard® Hematrace® Test Kit

4.0 PROCEDURE:

4.1 Label extraction tubes for identification.

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

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

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

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

5.0 COMMENTS:

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

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

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

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

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

5.6 Although most nonhuman species tested do not produce a positive result with the ABACard® Hematrace® test, some crossreactivity has been reported (i.e., other primates, weasel, 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).

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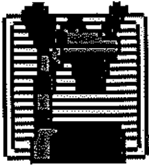

BI-108

8/28/01 | 8/30/01

SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION

1.0 BACKGROUND:

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

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

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

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2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

3% Ammonium Hydroxide (for aged stains)
Antisera
Agarose, E25
Glass Microscope Slide(s) or petri dish
GelBond®
Agarose Punch or equivalent (e.g., pipet and vacuum source)

Optional Staining: 1M NaCℓ; 0.1% Amido Black Stain and Destain solutions; filter paper

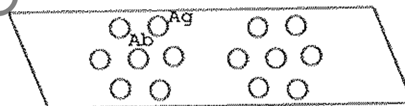
4.0 PROCEDURE:

4.1 In order for the agarose to sufficiently adhere to a microscope slide, GelBond® must be adhered to the slide and the agarose gel formed on top of it. Cut GelBond® to the approximate size of your microscope slide and adhere hydrophobic side to slide with a few drops of dH_2O.

4.2 Extract a small sample (e.g., $2mm^2$ bloodstain; $1cm^2$ stain of other suspected body fluids) in ~100 µℓ dH_2O (or 3% Ammonium Hydroxide for aged bloodstains). Bloodstain extracts should be somewhat dilute; straw-colored in appearance.

4.3 Prepare a sufficient quantity (~3 ml required to cover standard microscope slide; 2 tests/slide) of 1% agarose for the number of Ouchterlony tests to be performed. Carefully pour agarose onto hydrophilic side of the GelBond®. Allow solidification of gel.

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



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

4.5 Allow immunodiffusion to take place overnight, at room temperature, in a moisture chamber (enclosed vesicle with dH_2O-moistened paper towel or filter paper).

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4.6 Precipitin bands are best viewed with strong backlighting against a dark background. The immunodiffusion gel may be soaked, dried and stained for enhanced visualization.

4.7 (Optional Staining)

4.7.1 Soak immunodiffusion gel in 1M NaCl for ≥ 6 hours to remove uncomplexed proteins.

4.7.2 Rinse the gel in dH_2O for ~5 minutes; dampen two pieces of filter paper with dH_2O and place on top of gel. Dry gel in oven at 56°C-65°C for ≥ 20 minutes.

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

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

5.0 COMMENTS:

5.1 Coomassie Blue (Brilliant Blue R; 0.2%) may also be used in staining.

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

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

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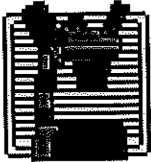

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2/4/2002	2-4-03
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BCIP TEST FOR ACID PHOSPHATASE

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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 10-20µℓ extract where appropriate) and use a pre-moistened swab for a negative control.

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

4.4 Incubate for 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.

5.3 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, where necessary, 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 BCIP Reagent and analyzed as above.

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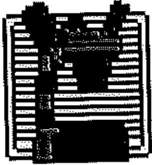

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2/4/2003	2-4-03
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BRENTAMINE TEST FOR ACID PHOSPHATASE

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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 10-20µℓ 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.

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SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION

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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. Remove supernatant as needed for AP screening (BI-112; BI-114) and p30 detection (BI-120), etc.

4.3 At this point, additional volume (e.g., 500µℓ), agitation, vortexing and brief sonication may be used to assist in removing sperm/cellular material from substrate. Remove the substrate and centrifuge the extract for ~5 minutes at ≥ 2,500 rpm.

4.4 Carefully remove all but ~30-50µℓ of supernatant without disturbing the pellet, then resuspend and use 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).

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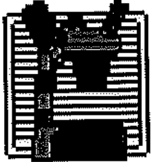

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8/28/01	8/30/01
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SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION

1.0 BACKGROUND:

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

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

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2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 The ~50µℓ of sample extract remaining after centrifugation and supernatant removal (BI-116) is mixed well and deposited on a microscope slide and allowed to dry (this process may be expedited by use of a slide warmer or oven at ~37°C).

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

4.0 PROCEDURE:

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

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

4.5 Allow the slide to dry and (optional) apply mounting medium and a cover-slip prior to microscopic examination.

4.6 Scan the slide on ≥200X magnification. Sperm heads will retain the red stain, while the tails will appear green. Use 400X magnification if necessary to verify sperm morphology.

4.7 Documentation in notes should include the following:

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

4.7.2 An estimate of the number of sperm seen per field (e.g., 12/slide; 0-1/200X; 3-5/200X; 5-10/200X; >10/200X etc.).

4.7.3 A description of the number and type of epithelial cells present (e.g., rare, occasional, few, moderate, many and nucleated [NECs] or anucleated [ANECs]. A scientist may also note whether or not there are large squamous epithelial cells.

4.7.4 If the situation arises in which there are only one or two sperm heads, a single intact sperm, or a few sperm head of questionable morphology, a second qualified scientist <u>must</u> verify the identification.

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

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

5.0 COMMENTS:

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

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

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1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABAcard® p30 Test Kit

4.0 PROCEDURE:

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

4.2 Add 10µℓ of each sample 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.

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5.0 **COMMENTS**:

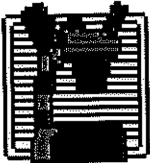
5.1 Samples must be at room temperature for the test.

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-122	
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AMYLASE TEST (PHADEBAS)

1.0 BACKGROUND:

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

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

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

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

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

Phadebas Amylase Test directions for use, Pharmacia AB, Uppsala, Sweden, 1994.

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2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Phadebas Tablets
0.5N NaOH
12x75mm tubes
Corks for tubes **or** parafilm™ or equivalent

4.0 PROCEDURE:

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

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

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

4.4 Examine tubes and record color. The intensity of the blue color may be graded as 1^+-4^+. For reporting, see **5.1**.

5.0 COMMENTS:

5.1 Positive controls should include 1:100 and 1:500 dilutions of fresh saliva as well as an extract of a known saliva stain. This stain might serve as a more appropriate control if it was prepared by licking the cloth instead of depositing liquid saliva. If the blue color of a sample is as darker or darker than that of the 1:100 control, it is an indication of an elevated level of amylase and is reported as 'detected the presence of saliva'. If the blue color of a sample is between that of the 1:100 and 1:500 controls, it is reported as 'indicating the possible presence of saliva'. If the blue color of a sample is lighter than the 1:500 control, there is no demonstration of an elevated amylase level and it is reported as 'inconclusive for the presence of saliva'. A sample that demonstrates absence of blue color consistent with the negative control is reported as 'did not detect the presence of saliva'. Note: negative samples (like the control) may have a very slight blue tint and not appear perfectly clear.

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

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

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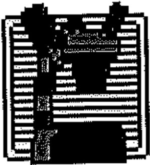

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AMYLASE TEST (STARCH IODIDE)

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1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

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

4.2 Prepare a 1% agarose/0.1% starch gel by dissolving 100mg of agarose and 10mg of soluble starch in 10mℓ of the Amylase Diffusion Buffer. Pour the gel into a (~9cm) petri dish, allow it to cool and punch wells ~2 mm in diameter, and at least 1.5cm apart, into the gel.

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

4.4 Mark petri dish for orientation and document sample placement.

4.5 Cover petri dish and allow diffusion overnight at 37°C.

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

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

5.0 COMMENTS:

5.1 Positive controls should include 1:100 and 1:500 dilutions of fresh saliva as well as an extract of a known saliva stain. If the clear zone of an extract ≥ that of the 1:100 control, it is an indication of an elevated level of amylase in the extract and is reported as 'detected the presence of saliva'. If the clear zone of an extract is between that of the 1:100 and 1:500 controls, it is reported as 'indicating the possible presence of saliva'. If an extract clears a zone smaller than the 1:500 control, there is no demonstration of an elevated amylase level and it is reported as 'inconclusive for the presence of saliva'. An extract that demonstrates no clearing zone is reported as 'did not detect the presence of saliva'.

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

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

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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.

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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.

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5.1 BLOODSTAINS/SALIVA SAMPLES (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., ~3mm²) 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**.

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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 ~1.5-3 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.

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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 H_2O
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.

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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**).

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5.4 EXTRACTION FROM SALIVA (ENVELOPE FLAPS AND STAMPS):

5.4.1 Cut an ~1.0cm^2 section into small pieces (note: steam may be used to release flap/stamp prior to extraction) and place in 1.5mℓ tube. Add 1.0mℓ of sterile water and incubate at 4°C for ≥5 hours (may be left overnight). **Steam may be used prior to cutting of envelope/stamp.**

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.

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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.

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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.

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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.

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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).

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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).

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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) or those that necessitate clean-up. Microconcentration of samples with high DNA concentrations will be performed separately from those with low DNA concentrations.

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).

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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).

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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.

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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**.

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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**.

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9.0 Comments:

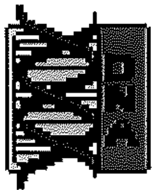
9.1 These methods employ the use of phenol that is a strong organic acid and may cause severe burns in addition to other effects. All operations with these chemicals should be performed in the hood with appropriate protective gear (gloves, lab coat and eyes protected by hood shield or goggles).

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.



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DNA QUANTIFICATION: QUANTIBLOT

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1.0 BACKGROUND:

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

"A Rapid Chemiluminescent Method for Quantitation of Human DNA," Walsh, P.S., et al, Nucleic Acids Research, Vol. 20, pp. 5061-5065.

"A Simple and Sensitive Method for Quantifying Human Genomic DNA in Forensic Specimen Extracts," Waye, J.S., et al, BioTechniques, Vol. 7, No. 8, 1989, pp. 852-855.

"The Convertible® Filtration Manifold System Instruction Manual," GIBCO BRL Life Technologies, Inc.

QuantiBlot™ Human DNA Quantitation Kit protocol, Perkin Elmer.

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Slot Blot Apparatus
Orbital Shaker
Shaking Waterbath
Vacuum Source
Spotting Solution (QSS)
Hybridization Solution (QHB)
Wash Solution (QWS)
Citrate Buffer (QCB)
Hybridization Container
Kodak Image Station
QuantiBlot™ Kit
Nylon Membrane
30% Hydrogen Peroxide
PCR-TE
Chemiluminescence Reagents

4.0 PROCEDURE:

4.1 PREPARATION OF DNA STANDARDS:

4.1.1 Label 8 sterile microfuge tubes A through H.

4.1.2 Mix Standard A thoroughly by vortexing, pulse-spin and transfer 40µℓ to the 'A' tube.

4.1.3 Dispense 20µℓ of PCR-TE into tubes B-H.

4.1.4 Prepare a serial dilution series by mixing and subsequent 20µℓ transfers from tubes A through G. H will contain PCR-TE only and serve as a negative control. Store standards at -20°C between uses. The dilution series consists of 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng in 5µℓ, respectively. New dilution series must be run with old prior to use.

4.2 IMMOBILIZATION OF DNA:

4.2.1 Fill out slot-blot form (Form 204-BI).

4.2.2 Cut a piece of positively charged nylon membrane (e.g., Biodyne® B; ~7.5 x 11.5 cm.) marking a corner for orientation by cutting it off. Incubate the membrane in ~100mℓ of spotting solution for 1-30 minutes at RT on an orbital shaker.

4.2.3 Using a microtiter plate, prepare the DNA standards, and samples by combining 5µℓ with 150µℓ of spotting solution (w/o stain). Use 1.25ng previously quantified 9947A as control.

4.2.4 Using forceps, place the pre-wetted membrane on the gasket of the slot-blot apparatus. Place the top plate of the slot blot apparatus on top of the membrane and turn on the vacuum source and the clamp vacuum (the sample vacuum should be in the 'off' position. Apply uniform pressure across top plate to ensure tight seal.

4.2.5 Carefully pipet samples into appropriate slot-blot wells, avoiding bubbles and contact with the membrane.

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4.2.6 Once all samples are in wells, slowly turn on the sample vacuum allowing them to be drawn onto membrane for ~30 seconds. Release the clamp vacuum, disassemble the slot-blot apparatus, remove the membrane and proceed.

4.3 DNA HYBRIDIZATION:

4.3.1 Pre-hybridization: Place the membrane and ~100mℓ pre-warmed hybridization solution in the plastic tray. Add ~5mℓ of 30% H_2O_2. Place the lid on the plastic tray. Rotate in a 50°C (±1°C) water bath (50-60 rpm) for 15 minutes (±2 minutes). Pour off the solution. Briefly rinse membrane in a small amount of pre-warmed hybridization solution. Pour off the solution.

4.3.2 Hybridization: Mix 30mℓ of hybridization solution and 20µℓ of QuantiBlot D17Z1 probe in a disposable plastic tube. Pour this solution into the tray containing the membrane. Place the lid on the tray. Rotate in a 50°C (±1°C) water bath (50-60 rpm) for 20 minutes (±2 minutes). Pour off the solution.

4.3.3 Rinse the membrane briefly in 50-100mℓ of pre-warmed wash solution. Pour off the solution.

4.3.4 Stringent Wash/Conjugation: Mix 30mℓ of the pre-warmed wash solution and 90µℓ of Enzyme Conjugate: HRP-SA in a disposable plastic tube. Pour this solution into the tray containing the membrane. Place the lid on the tray. Rotate in a 50°C (±1°C) water bath (50-60 rpm) for 10 minutes (±2 minutes). Pour off the solution.

4.3.5 Rinse the membrane 2-3 times (for 30-60 seconds) with approximately 100mℓ of pre-warmed wash solution.

4.3.6 Add approximately 100mℓ pre-warmed wash solution to the tray. Place the lid on the tray. Rotate at room temperature on an orbital shaker (100-125 rpm) for at least 15 minutes. Pour off the solution.

4.3.7 Rinse the membrane briefly (1-2 times) with 50-100mℓ of citrate buffer. Pour off the solution.

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4.4 DETECTION:

4.4.1 Add prepared chemiluminescence reagent (see **5.2**) to membrane, covering completely (5-10mℓ) and rotate for 1-5 minutes per manufacturer's instruction. Alternatively, the chemiluminescence reagent may be placed directly on the Image Station platen and the membrane placed (DNA side down) on top of it.

4.4.2 Proceed with membrane processing on the Kodak Image Station (BI-206).

4.5 RE-HYBRIDIZATION OF MEMBRANE:
It may sometimes be desirable, due to lack of signal or high blot background, to re-hybridize the same membrane rather than begin and entirely new slot-blot.

4.5.1 To strip the DNA probe off the blot membrane, Heat 150mℓ of the Wash Solution to approximately 90°C in a glass container and pour it into a tray containing the membrane.

4.5.2 Rotate the tray on an orbital shaker at room temperature for 20 minutes.

4.5.3 Remove the membrane from the wash solution, return to step **4.3.2** and continue the protocol from that point.

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5.0 **COMMENTS:**

5.1 Do not allow membrane to dry-out during the process.

5.2 Chemiluminescence reagents used with the Quantiblot kit (e.g. NEN Western Blot Chemiluminescence Reagent Plus, or ECL reagents from Boehringer Mannhein and Amersham Pharmacia Biotech) have two components that need to be mixed, typically in equal volumes, just prior to use. Check product insert for information.

5.3 Clean the slot-blot apparatus thoroughly (e.g., Neutrad).

5.4 In the event that the Kodak Image Station, or other mechanism of detecting chemiluminescence, is unavailable, the scientist will revert to colorimetric detection previously used. Follow protocol modifications per manufacturer's instructions.

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DNA QUANTIFICATION: KODAK IMAGE STATION

1.0 BACKGROUND:

When quantifying DNA, the detection method chosen may involve light emission. As an alternative to recording the light emission on film , the Kodak Image Station may be used. The image station employs a CCD camera to capture a digitized record of light intensity. Software is then used to detect bands from the slot blot and compare their intensity.

Kodak Digital Science™ Image Station 440_{CF} User's Manual
Kodak 1D Image Analysis Software User's Manual

2.0 SCOPE:

To provide a reliable method for quantification of DNA on chemiluminescent blots.

3.0 EQUIPMENT/REAGENTS:

Kodak Image Station 440 CF
Kodak 1D Image Analysis Software
Chemiluminescent blot

4.0 PROCEDURE:

4.1 IMAGE CAPTURE:

4.1.1 Access Kodak 1D program from computer desktop (Perform these first two steps while blot is washing).

4.1.2 Click on Capture IS 440 CF button at upper left and verify camera settings: f-stop=1.2, zoom=~25, no filter, (i.e. "0").

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4.1.3 Place the membrane, DNA-side down, directly on the platen of the image station (this may be performed by placing it in a puddle of chemiluminescence reagent and incubating directly on platen, see BI-202, or BI-204).

4.1.4 Click on 'Preview', click off 'x and y binning', click on 'expose'. Center membrane on platen using the image on the monitor and click 'stop'. Close lid of platen (wait for chemiluminescence incubation if performing on platen).

4.1.5 Deselect 'preview' and set bar sliders to 3 x "x" minute exposures ("x" will vary with chemiluminescent reagent used and slot-blot; typically ~"7" minute exposures is a good starting point) leave x and y binning on, and click 'expose'. It will display image after the first 7-minute capture and add to it after the second and third exposures. Status of time elapsed is shown at bottom of screen. Capture will stop automatically.

4.1.6 Hit 'submit' at the lower left and an image field correction box appears on screen. Confirm camera settings listed are those you verified in **4.1.2**, and check 'apply lens correction' box then, 'OK'.

4.1.7 To save the image as a file, perform a 'save as' file command. The file information box that appears is an annotation that may be left blank or used for comments. It may be changed at any time. Click 'OK'.

4.1.8 Use the slider at the bottom of the image to adjust its size (e.g., 1.5X) and use the 'edit menu' to rotate the image.

4.1.9 Press 'CTRL-2' to bring up exposure adjustment window and click the 'max button' and select the most intense band on the slot-blot. Click the 'min button' and select an area on the slot-blot that has the darkest background that is above the lightest band you wish to quantify. Adjust with sliders if necessary.

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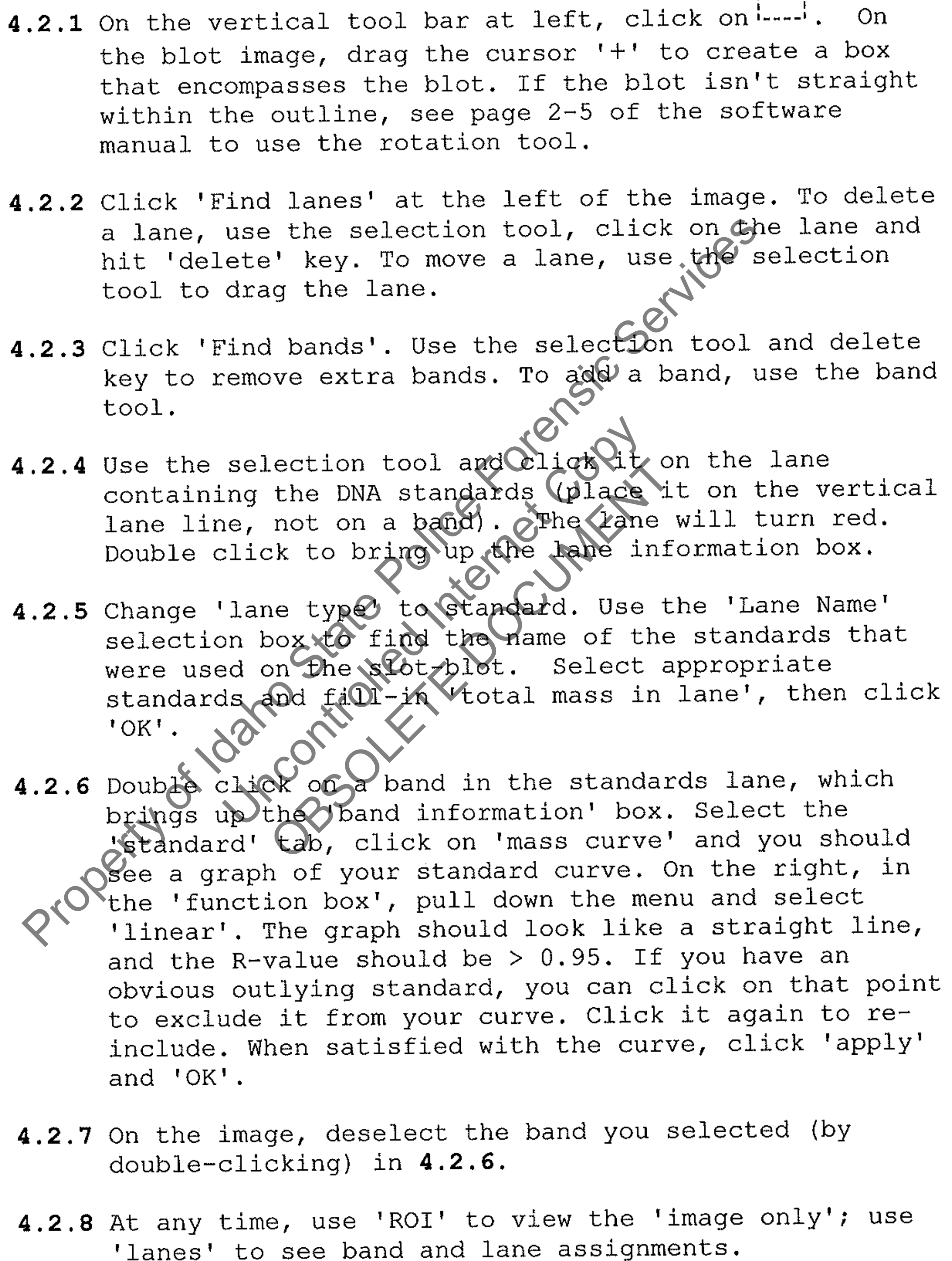

4.2 BAND LOCALIZATION AND QUANTIFICATION:

4.2.1 On the vertical tool bar at left, click on [dashed box icon]. On the blot image, drag the cursor '+' to create a box that encompasses the blot. If the blot isn't straight within the outline, see page 2-5 of the software manual to use the rotation tool.

4.2.2 Click 'Find lanes' at the left of the image. To delete a lane, use the selection tool, click on the lane and hit 'delete' key. To move a lane, use the selection tool to drag the lane.

4.2.3 Click 'Find bands'. Use the selection tool and delete key to remove extra bands. To add a band, use the band tool.

4.2.4 Use the selection tool and click it on the lane containing the DNA standards (place it on the vertical lane line, not on a band). The lane will turn red. Double click to bring up the lane information box.

4.2.5 Change 'lane type' to standard. Use the 'Lane Name' selection box to find the name of the standards that were used on the slot-blot. Select appropriate standards and fill-in 'total mass in lane', then click 'OK'.

4.2.6 Double click on a band in the standards lane, which brings up the 'band information' box. Select the 'standard' tab, click on 'mass curve' and you should see a graph of your standard curve. On the right, in the 'function box', pull down the menu and select 'linear'. The graph should look like a straight line, and the R-value should be > 0.95. If you have an obvious outlying standard, you can click on that point to exclude it from your curve. Click it again to re-include. When satisfied with the curve, click 'apply' and 'OK'.

4.2.7 On the image, deselect the band you selected (by double-clicking) in **4.2.6**.

4.2.8 At any time, use 'ROI' to view the 'image only'; use 'lanes' to see band and lane assignments.

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4.2.9 On the lower left of the image, use 'options' pull-down menu to select 'lane analysis data', to view the quantification data generated. Values in red are outside the linear range of your standard curve.

4.3 PRINTING:

4.3.1 Use the 'ROI' to view the image only and use File: Print. This brings up the Print box on the computer screen. Check the □ for Image, and deselect any other boxes. Go to 'image layout' to see how it will look, then select 'OK'. In the printer screen, click 'Setup', 'Properties', 'Graphics and click on 'color'. Then choose the 'print in black and white only' option, and then back out to the print window by selecting 'OK' and then 'print'. This process will print the image only without the lane and band assignments, allowing a view of faint bands that might otherwise be masked by the band markers.

4.3.2 To print the lane analysis table and a copy of the image showing the band assignments: Use the 'lanes' tab to view image and from the print window select 'lane analysis', which will bring up 'lane layout', which you select. From the Data pull-down menu, select 'one page summary (with image)'. Print.

Remove membrane from image station; gently wipe platen and clean it with water and Kimwipes.

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5.0 COMMENTS:

5.2 For Quantiblot®, using 180μℓ of enzyme-conjugate (as is done in colorimetric development) may improve sensitivity.

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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

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2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

4.1.1 Based upon the quantity of DNA isolated and its initial concentration, the scientist should have all samples at an optimal concentration for amplification (e.g., 0.1ng/µℓ-0.4ng/µℓ). It is also convenient to have all samples that are to be amplified at the same time to be at the same concentration if possible for ease in the preparation of PCR Master Mix and reaction additions. For those samples that were deemed to be <1ng (or not detected at all), at a minimum, the sample must be concentrated and the entire sample amplified. Where possible, it is preferable for additional sample to be extracted, quantified and combined prior to amplification.

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), 4-6ng template should be used with offender sample runs as the amplification cycle number is reduced for those samples.

4.2 AMPLIFICATION SET-UP:

4.2.1 Amplification reaction set-up is to be performed in the designated biological hood using those dedicated pipets and tips (see 5.1 comments).

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.

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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 dH20 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.

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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.

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

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

60°C for 45 minutes, then:

4°C soak

4.3.1.2 For non-quantified DNA use 'pp16buccal'; the cycling conditions are as follows:

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

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

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

60°C for 45 minutes, then:

4°C soak

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

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

60°C for 45 minutes, then:

4°C soak

If, from the data generated on the 310 Genetic Analyzer, it is determined that the signal for a sample falls below a 100-rfu threshold but in other respects appears to be good data, the scientist may remove 10µℓ of the PCR reaction, transfer it to a new microAmp tube and run the above cycling program. The negative control and reagent blank should be run through the same process. The positive control will 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. For evidentiary forensic samples, 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. Prior technical leader approval is required for this process. See BI-210 4.4.2.5 RFU Threshold for additional information.

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4.4 <u>AMPLIFIED DNA PRODUCT</u>:

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 **<u>ONLY</u>** stored in the Amp/PostAmp room.

4.4.2 At a point in time after STR analysis is completed (i.e., case has been reviewed and report approved or CODIS data has been reviewed and approved for upload), the amplified product will be sealed in a biohazard container, transported directly to the autoclave, sterilized and disposed of with other biohazardous material.

5.0 COMMENTS:

5.1 Clean surfaces with freshly made 10% bleach solution or Dispatch® prior to set-up. UV light exposure of 20-30 minutes may be used in addition to the cleaning.

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

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

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 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).

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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 to produce STR profiles from evidentiary material and convicted offender samples for entry into CODIS.

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

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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.

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

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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 per analyst's discretion] but a 3 sec. inj. time for single-source samples estimated at ≥ 1ng and 5 sec. for samples < 1ng generally produce good results). Varying injection times beyond 5 seconds must be noted on the GT electropherogram and should be reserved for limited single-source forensic or CODIS samples.

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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 0.25μℓ-1.0μℓ 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 Genescan® Injection List (65%) for CODIS runs.

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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 an analysis computer. After analysis and review are complete, one copy of the analyzed run folder will be stored on Fleetwood (to be archived to CD) and one will remain on analysis computer until CD archiving has been completed. The Run Folder on the instrument computer (Mendel or Ros) may be deleted at this point.

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. Save projects as Matrix MM/DD/YY or CODIS Run (or CODIS QC) YYYY-#

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.

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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.

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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. General 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 threshold in all colors. Rfu threshold may be raised in Blue, Green and Yellow for Allelic Ladder or CODIS samples only. Rfu threshold may be lowered to 100 rfu at the analyst's discretion and must be noted on the GT electropherogram. Lowering of rfu threshold below 100 rfu (to ≥50) requires **PRIOR** Technical Leader approval **AND** necessitates that peaks are a minimum of 4-fold localized baseline background. Peaks below 50 rfu are deemed inconclusive. Lowering of rfu threshold should be used with discretion and generally reserved for samples that would not benefit from re-extraction/re-analysis.

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. ldr parameters or 100rfu).

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.

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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"YYYY"-# or other appropriate name for QC, validation or research project(s).

4.2.4.7 For 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

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printed for the case file or CODIS binder. Once the analysis is completed and has been reviewed, a copy of the analyzed folder will be stored on the analysis computer and Fleetwood until burned to a CD for archival purposes. Case-specific CDs will be made for discovery upon requested.

4.3 ALLELE ASSIGNMENT: GENOTYPER®

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The PowerTyper™ 16 Macro is used within Genotyper® 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 (typically Amelogenin peaks), the 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. These changes or "clicking' off of any peaks, as well as the presence of bleed-through must be noted on the GT electropherograms.

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'; use locus stutter values in Appendix A to assess potential contribution to peaks in stutter positions), 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 (generally CODIS macro). The table will be exported to Excel, and may be used to generate a cmf file (typically CODIS runs) for CODIS import; the table will be printed for the case file or CODIS binder.

4.3.8 Print (at 75%) the Genotyper® Plots for case files and CODIS binder. The electronic Genotyper® data is stored and burned for archiving as described above.

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 (or CODIS QC RUN) YYYY-#" Genotypes or similar designation.

4.3.10 Samples demonstrating an off- ladder (< or > smallest or largest ladder allele, respectively) or microvariant (alleles with incomplete repeats) allele(s) should be re-analyzed for verification where necessary (e.g., evidentiary profile in nonsuspect case, CODIS sample). 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. Note that the nomenclature for upload to NDIS may necessitate a change in allele designation.

4.4 STR INTERPRETATION GUIDELINES AND STATISTICAL ANALYSES

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4.4 STR INTERPRETATION GUIDELINES AND STATISTICAL ANALYSES

4.4.1 CONTROLS

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

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

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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 beyond that point). 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 will be examined for the presence of the same peak(s). It is possible, since this control is processed last and its tube deliberately left open during the amplification set-up (to demonstrate maximum contamination potential), that it would be the only sample affected.

If extraneous peaks appear <u>only</u> in this control, the data for other samples associated with that amplification need <u>not</u> be deemed inconclusive. This occurrence should be documented and the scientist's determination (and basis for it) documented in the case file.

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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 must be run with every forensic extraction set (will generally be run with CODIS extractions also). 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 (4-6ng) DNA may be used. Failure of the blind control, if isolated to that sample, will not deem other samples inconclusive.

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4.4.2 RFU THRESHOLD:

4.4.2.1 For single-source forensic, (sufficient in quantity and condition) CODIS samples and reference standards (excluding autopsy samples that may be degraded or of limited quantity), a minimum of 100 rfu must be achieved for data acceptance. If necessary, go back in the process as follows: repeat injection (changing injection time; 3-10 seconds allowable range), or perform re-analysis (i.e., changing amount of PCR added for fragment analysis), or re-amplification (increase DNA template), or re-extraction.

4.4.2.2 For minor mixture components (or low-copy single-source forensic samples), a threshold of 50 rfus may be used (see 4.2.3 Peak Detection). However, depending on signal/baseline may be deemed inconclusive.

4.4.2.3 Peaks below 50 rfu may (based on data obtained-peaks must be ≥4X localized background) 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 (see **4.2.4.5**) or repeated at the scientist's discretion. Application of a CC matrix may be necessary for higher rfu samples.

4.4.2.5 The additional cycle (+3AMP) option may <u>only</u> be used with prior technical leader approval and when the following conditions are met: 1) most or all of a sample has been consumed, 2) the 100-rfu threshold has not been met but most alleles (≥ 70%) are ≥50 rfu and appear relatively balanced within a locus. Additional cycling option may also be used for CODIS samples that meet condition 2 and have been extracted ≥2 with similar results. Final data (following +3 AMP) must exceed 100 rfu at all loci for CODIS samples and any loci used for statistical inclusion of forensic samples.

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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 demonstrate only two alleles but have a heterozygous ratio of <70% may also be indicative of a mixture. However, if data are obtained from multiple loci, a scientist should expect to see this or other mixture indications (> 2 alleles) at additional loci.

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

4.4.3.4 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 may be predominant (i.e., the "highest rfu peak" in the 3-peak 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".

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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 DNAView 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 $f_{(pp)} = p^2 + p(1-p)\theta$, where $\theta = 0.01$ except where small isolated populations (e.g., Native Americans) may be relevant, in which case, $\theta=0.03$.

4.4.4.4 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).

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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 non-genetic 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 denominator of the RMP obtained for an evidentiary item, from the analysis of several polymorphic STR loci, exceeds the population of the world several-fold. However, no reasonable individual would make the assertion that every individual in the world need be considered a potential DNA source in the context of a given case. **'SOURCE ATTRIBUTION'** (see Budowle, B. et al, Source Attribution of a Forensic DNA profile. *Forensic Science Communications*. 2(3) July 2000) is the result of a statistical approach to 'operationally' define uniqueness (assess whether a given multi-locus DNA profile could be considered unique for a given case).

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The equation $p_x \leq 1-(1-\alpha)^{1/N} \approx \alpha/N$, is used to determine maximum RMP (p_x) that would support 'source attribution' for a relevant population sample size (N) and selected confidence limit (i.e., $\alpha=0.01$; $1-\alpha=99\%$ confidence).

The FBI has selected an upper confidence limit (UCL) of 99% ($\alpha=0.01$) and an "N" equivalent to the U.S. population (2.6×10^8 pre-2000 census). This is reasonable as the FBI performs casework for jurisdictions all over the country and this calculation would provide a uniform approach to be used regardless of jurisdiction. For these figures, an RMP of $>3.9\text{x}10^{-11}$ (or less than 1 in $2.6\text{x}10^{10}$) 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 $2.6\text{x}10^{11}$ for the reporting of source attribution.

In Idaho, using 2000 consensus figures, an operational population (N) of $1.6\text{x}10^7$ has been selected (representing the sum of the populations of Idaho and the six surrounding states: ID=$1.3\text{x}10^6$; MT=$0.9\text{x}10^6$; NV=$2.0\text{x}10^6$; OR=$3.4\text{x}10^6$; UT=$2.2\text{x}10^6$; WA=$5.9\text{x}10^6$ and WY=$4.9\text{x}10^5$). Therefore, an RMP of less than 1 in $1.6\text{x}10^{10}$ (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).

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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.

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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:

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 a sample has not been received, the submitting agency should be notified.

4.1.2 Where possible, compare the DNA sample card information to that of the Collection Report Form to ensure accuracy. Data for a sample may be entered in absence of a DNA Collection Report form, however, an IDOC# is necessary for data entry into STiMAS. Contact necessary IDOC personnel for a number if one has not been recorded. The DNA Collection Report Form will be destroyed after STiMAS data entry.

4.2 STiMAS ENTRY (Pre-entry Search):

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4.2.1 Prior to data entry for any new sample, a database search is performed to eliminate duplicate sample entry and processing. *Log on* to the CODIS workstation computer and open the STiMAS database program. Generally, the screen will display the "Convicted Offender" application with menu choices visible in a box to the right.

4.2.2 'Browse Offenders' is the STiMAS application search function. Access to this function may be gained through 'Browse' under the 'Offender' pull-down menu or by double-clicking on this choice in the list.

4.2.3 The 'Browse Offenders' screen allows for searches based on any data entered. A duplicate sample search will be performed using both the required IDOC# and the Last Name fields as follows:

1) Select the 'Filter' radio button.
2) Select Last Name for 'Field Name', 'LIKE' for condition (This is to compensate for spelling variations when used in combination with the wildcard '*" in the 'value field).
3) Type the appropriate letters of the offender's last name in the 'value' field.
4) Mark the box on the second line indicating an additional parameter to be searched and select 'OR' to ensure that unless there is an error in both the Last Name and IDOC# entries, a duplicate will be identified.
5) Select 'Corrections' for 'Field Name', '=' for condition and type the IDOC number into the 'value' field.

4.2.4 Once all the parameters have been defined and the data entered, clicking on the 'Filter' button will initiate the search.

4.2.5 If the search returns 'The search found 0 record(s) that met the criteria', the data for the new sample may be entered into the STiMAS Offender Submission screen (see 4.3).

4.2.6 If a record(s) is returned that meets the criteria, the data is examined and compared with the new sample received. If the sample is determined to be a duplicate, the sample folder is marked by hand 'Duplicate of IDYYYY###### and is physically attached to the duplicate that has already had sample information entered into STiMAS. Duplicates may be destroyed after data for initial sample has been verified for upload to CODIS. This will be documented on the folder of original sample (Include date of sample receipt, sample destruction and initials of individual affecting destruction).

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4.3 STiMAS ENTRY (New Sample Data Entry):

4.3.1 Once it has been verified that the sample is not a duplicate, enter information from the sample card and/or DNA Collection Report form into the appropriate fields. Double-check all information **BEFORE** saving the sample submission form. It is particularly important that the 'Submission Date' (this is date received in ISP Forensic Services) field is correct (in particular the year) as the assigned CODIS identifier is an autonumber that is generated upon saving and is based, in part, on this date. Click on 'Save' button.

4.3.2 After saving, print the corresponding labels by clicking on the 'Barcode Labels' button. Print two labels. Place a barcode label on the DNA sample folder and insert behind or attach a second label to the FTA card envelope. This label will be placed on the FTA card at the time of DNA analysis. Update back-up STiMAS copy.

4.3.3 Place the DNA sample card in one of the secure filing cabinets.

BI-302	
2/4/2003	2-4-03
[illegible]	RDS

CODIS SAMPLE DATA ENTRY AND UPLOAD

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. Accurate data entry for upload to SDIS (State DNA Index System) and NDIS (National DNA Index System) is essential.

2.0 SCOPE:

To provide a mechanism to ensure accurate data entry for all offender and forensic database samples.

3.0 EQUIPMENT/REAGENTS:

CODIS Computer Workstation

Allele Tables and/or CMF files generated from sample analyses.

CODIS Training Manual

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4.0 PROCEDURE:

4.1 SAMPLE DATA VERIFICATION:

4.1.1 Genotypic data (allele calls) are checked for accuracy and verified during the CODIS/Casework Review process and documented on the appropriate form (Form 306-BI and Form 214-BI, respectively). This is accomplished by examination of the computer generated allele tables (or the cmf [common message format] or equivalent generated from the allele tables).

4.1.2 Additionally, when 'PCR Analysis' is used to enter individual sample data (generally forensic samples) verification of 1st and 2nd 'reader' is automatically achieved prior to transfer to LDIS and subsequent upload to SDIS/NDIS.

4.2 SAMPLE DATA ENTRY IN CODIS:

4.2.1 Once sample data accuracy (forensic and offender) has been verified, data may be entered into the CODIS database either by use of the 'PCR analysis' module to enter specimen ID/info and allele calls for individual samples or by using the 'Import' function for the entry of batches of data contained in a cmf (or equivalent) file. Refer to the CODIS Training Manual and course documentation for specific steps in accomplishing these tasks.

4.2.2 .A second 'reader' must also log on and enter allelic data for the individual samples enter through 'PCR Analysis'. A 'check' indicates agreement between readers at individual loci and discrepancies in entry must be rectified before transfer to LDIS. The second reader will perform the transfer after all readings are determined to be in agreement.

4.2.3 When using the 'Import' function, the scientist will open the appropriate cmf (or equivalent) batch file select 'validate import'. This will ensure that any typos or inconsistencies (i.e., variant allele definitions/equivalencies) will be identified prior to import and may be corrected. Once the batch file has been validated for import (corrections performed if necessary), 'import' is selected by the scientist and the process of importing the batch file data into LDIS will commence. After data has been is in LDIS, by either mechanism, it is available for upload to SDIS.

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4.3 CODIS DATA UPLOAD:

4.3.1 SDIS In the CODIS Local Administration module, select 'Bulk Upload to SDIS' from the System Administration pull-down menu. The data uploaded will then be sent as a message attachment to SDIS via DNACOMM. Double-clicking the message in DNACOMM will 'process' the upload at SDIS. If any 'candidate matches' are identified, a match message will appear in DNACOMM and they will also be reflected in Match Manager. For hit verification see BI-306.

4.3.2 NDIS There are various reasons that some samples present at SDIS should not be uploaded to NDIS (e.g., juvenile samples not accepted at NDIS). Prior to NDIS upload, these samples will be selected in Specimen Manager and 'unmarked for upload'. Generally speaking, an incremental upload will be performed. In Specimen Manager, 'incremental upload' is checked on the 'upload' pull-down menu and 'send upload' is selected. The upload is sent to NDIS as a message attachment via DNACOMM. . If any 'candidate matches' are identified at NDIS, a match message will appear in DNACOMM and they will also be reflected in Match Manager. For hit verification see BI-306.

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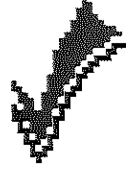
5.0 COMMENTS:

5.1 Refer to CODIS Training Manual and course documentation for more specifics if necessary.

5.2 The CODIS software is redundant and there is generally more than one way to accomplish many tasks. Using a mechanism other that listed here is acceptable.

5.3 The CODIS software is updated periodically and any necessary changes in procedure provided with new updates supercedes those in procedures written prior to update, if appropriate.



Form 404-QC

FORENSIC BIOLOGY WEEKLY 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/RO SYSTEM				
EYE WASH STATION				
AMP ROOM TEMP (AC controller)				
°C WATER BATH A (BCIP)				
°C WATER BATH B (SHAKER)				
°C WATER BATH C (REAGENTS)				
°C OVEN				
°C HEATING BLOCK				

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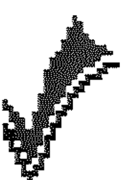

FORENSIC BIOLOGY MONTHLY QC

Form 406-QC

DATE												
BIOSAFETY HOOD												
AIR FLOW												
WIPE UV LIGHT												
CHECK PAPER												
AUTOCLAVE												
WIPE DOOR GASKET												
FLUSH STEAM GENERATOR												
CHECK STERILIZATION												
pH METER												
ARTEL PCS2												
CLEAN CENTRIFUGES												
CLEAN PIPETS												
PCR LAB CLEANED												

*Personnel should initial the duties they perform and date separately, if necessary.

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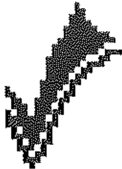

Form 408A-QC

FORENSIC BIOLOGY QUARTERLY QC

I. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 9700

DATE:
TESTED BY:
PROBE SERIAL # 6000029
THERMOMETER SERIAL # 00D400195

Temperature Non-Uniformity

SETPOINT VALUE	94 *C	37 *C
A1		
A12		
C4		
C9		
F4		
F9		
H1		
H12		
TNU AT 94°C: _____ PASS FAIL		
TNU AT 37°C: _____ PASS FAIL		

Calibration Verification

SETPOINT VALUE			PASS
A6	85°C	45°C	
			FAIL

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II. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 480

DATE:
TESTED BY:
PROBE SERIAL # P16944
THERMOMETER SERIAL # 1093237L

Temperature Uniformity (File 32)

SETPOINT VALUE	95 °C	40 °C	SETPOINT VALUE	95 °C	40 °C
A1			C6		
A3			C8		
A6			F1		
A8			F3		
C1			F6		
C3			F8		

II. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 480 (cont.)

95°C Readings

High - Low: (____) - (____) = ____ Acceptable value is < 1°C.

Average ____ Acceptable value is within ± 1°C of target temperature.

40°C Readings

High - Low: (____) - (____) = ____ Acceptable value is < 1°C.

Average ____ Acceptable value is within ± 1°C of target temperature.

Temperature Calibration Verification (File 33)

Observed °C at 95 (____) - 0.10 = (____) [Block Average]

Acceptable Range is 94.1-95.9°C

Observed °C at 40) (____) +0.01°C = (____) [Block Average]

Acceptable Range is 39.1 - 40.9°C

Diagnostic Files Quality Control Record

Test #1: Display Check ____

Test #4: Chiller Test _____ (0.85 - 1.90°/sec)

Prior to test #5 call up File #35 and run for 10 minutes.

Test #5: Sensor Test _____ (< 0.5°C)

Test #6: Overshoot _____ (<0.5°C)

Test #7: Undershoot _____ (<2.0°C)

III. Instrument Firmware and Macs HD Optimization

DATE:______

PERFORMED BY:_____

MENDEL: NORTON UTILITIES______
RE-BUILD DESKTOP______
RE-SEND FIRMWARE______

ROS: NORTON UTILITIES______
RE-BUILD DESKTOP______
RE-SEND FIRMWARE______

PLACE DOCUMENT IN PCR QC BINDER

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Form 414-QC

QC PCIAA

(Phenol:Chloroform: Isoamyl Alcohol 25:24:21

PCIAA LOT: ________________ **DATE RECEIVED:** ____________

SCIENTIST: ___________ **QC DATE:** ____________

Extract a single known (genotype) sample following appropriate organic procedure. Perform extraction, quantification, amplification and STR analysis as usual. Attach all appropriate process documentation including Genotyper® electropherograms.

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QA/QC PASSED: **YES** ☐ **NO** ☐

Comments:

Form 416-QC

QUANTIBLOT Kit QC

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# ____________

To check new Kit Lot, perform quantification procedure as usual. For samples, run standards from old and new kit as well as 0.5ng and 10ng of 9947A DNA. On Kodak Image Station, quantify with old standard and new. Print both sets of documentation and place in Forensic Biology QC Binder.

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

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Form 418-QC

ACES KIT QC

Scientist ____________________ ImageID ____________________

Date ______________

ACES™ Kit Lot# ______________ Pre-wetting Solution Lot# 2XSS __________

Membrane Source ________________ Lot# ________________

Denaturation/Spotting Solution Lot# DS __________

Neutralization Solution Lot# NS __________

ACES™ Final Wash Buffer Lot# __________

Chemiluminescence Reagents Lot# ______________

To check new Kit Lot, perform quantification procedure as usual. For samples, run standards from old and new kit as well as 0.5ng and 10ng of 9947A DNA. On Kodak Image Station, quantify with old standard and new. Print both sets of documentation. and place in Forensic Biology QC Binder.

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A1 STD 40NG	A2 STD 0.1NG	A3	A4	A5	A6
B1 STD 20NG	B2 STD 0.04NG	B3	B4	B5	B6
C1 STD 10NG	C2 STD ØNG	C3	C4	C5	C6
D1 STD 4NG	D2	D3	D4	D5	D6
E1 STD 2NG	E2	E3	E4	E5	E6
F1 STD 1NG	F2	F3	F4	F5	F6
G1 STD 0.4NG	G2	G3	G4	G5	G6
H1 STD 0.2NG	H2	H3	H4	H5	H6

Form 424-QC

QUARTERLY 310 QC RUN

SCIENTIST:________________ **QC DATE:**_____________

Once a quarter, 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-24.2, 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 assessed 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 CD.

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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.

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9.0 PROFICIENCY TESTING

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

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

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

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10.0 CORRECTIVE ACTION

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

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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.

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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).

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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.

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14.0 AUDITS

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

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

14.1 Every other year, the DNA audit must be an external audit. There are additional NDIS reporting requirements associated with these external DNA audits. Those requirements must be fulfilled in accordance with NDIS Operational Procedures.

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