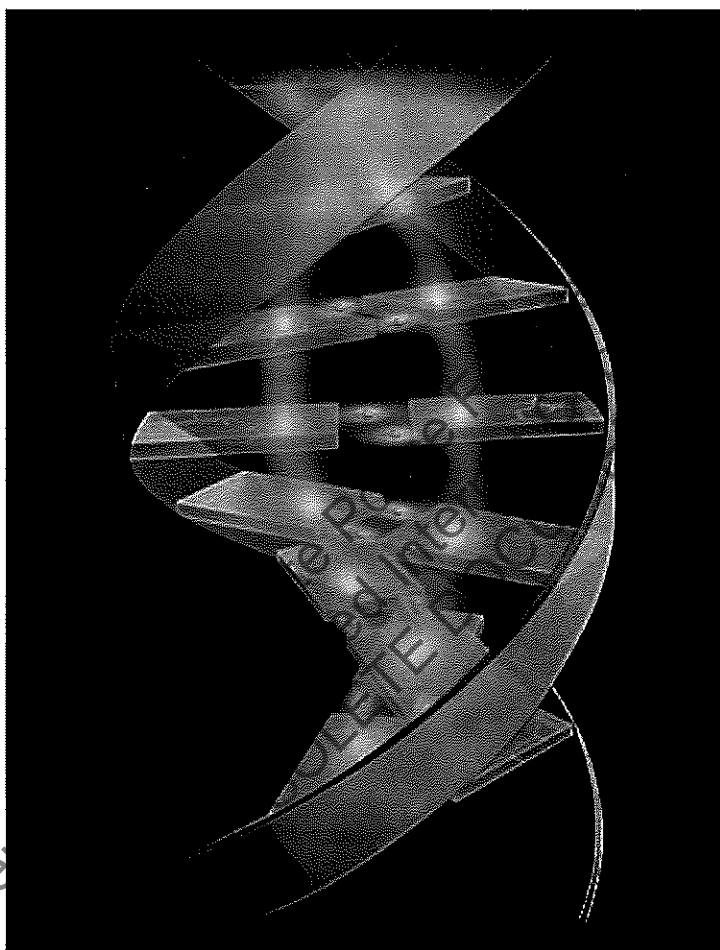


ISP FORENSIC BIOLOGY QA MANUAL



June 2003

APPROVED BY:

[Signature]
Biology/DNA Supervisor

[Signature]
Lab Improvement Manager

Date: 6/11/03

Date: 6-13-03

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.

Forensic Biology QA Manual/ Protocol Revision History
June 3, 2003

1st Revisions of Existing Protocols/Forms:

MBI-104 EXAMINATION OF EVIDENCE FOR BODY FLUIDS
BI-105 O-TOLIDINE TEST FOR BLOOD
BI-108 SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION
BI-118 SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION
BI-122 AMYLASE TEST (PHADEBAS)
BI-124 AMYLASE TEST (STARCH IODIDE)
BI-202 DNA QUANTIFICATION: QUANTIBLOT
BI-206 DNA QUANTIFICATION : KODAK IMAGE STATION
BI-302 CODIS SAMPLE DATA ENTRY AND UPLOAD
404-QC FORENSIC BIOLOGY WEEKLY QC
406-QC FORENSIC BIOLOGY MONTHLY QC
408A-QC FORENSIC BIOLOGY QUARTERLY QC

2nd Revisions of Existing Protocols:

BI-QA QUALITY MANUAL
MBI-100 EXAMINATION OF BLOODSTAINED EVIDENCE
MBI-200 INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSES
MBI-400 DOCUMENTATION IN FORENSIC BIOLOGY
BI-100 PROCESSING LIQUID BLOOD
BI-102 DNA PACKETS
BI-104 PHENOLPHTHALEIN TEST FOR BLOOD
BI-106 HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST
BI-112 BCIP TEST FOR ACID PHOSPHATASE
BI-114 BRENTAMINE TEST FOR ACID PHOSPHATASE
BI-116 SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION
BI-120 IDENTIFICATION OF SEMEN BY P30 DETECTION (ABACARD®)
BI-200 EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS
BI-208 STR AMPLIFICATION: PP16
BI-210 STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS
BI-301 CODIS SAMPLE RECEIPT AND STIMAS ENTRY

Protocols/Forms Removed:

414-QC QC PCIAA
416-QC QUANTIBLOT KIT QC
418-QC ACES KIT QC
424-QC QUARTERLY 310 QC RUN

Original protocols are archived with Laboratory Quality Manager.

Forensic Biology QA Manual/ Protocol Revision History
February 4, 2003

1st Revisions of Existing Protocols:

BI-QA QUALITY MANUAL (Revised with protocol changes/additions)
MBI-100 EXAMINATION OF BLOODSTAINED EVIDENCE
MBI-102 EXAMINATION OF EVIDENCE FOR SEMEN
MBI-200 INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSES
MBI-400 DOCUMENTATION IN FORENSIC BIOLOGY
BI-100 PROCESSING LIQUID BLOOD
BI-102 DNA PACKETS
BI-104 PHENOLPHTHALEIN TEST FOR BLOOD
BI-106 HUMAN BLOOD IDENTIFICATION USING ABACARD[®] HEMATRACE[®] TEST
BI-112 BCIP TEST FOR ACID PHOSPHATASE
BI-114 BRENTAMINE TEST FOR ACID PHOSPHATASE
BI-116 SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION
BI-120 IDENTIFICATION OF SEMEN BY P30 DETECTION (ABACARD[®])
BI-200 EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS
BI-208 STR AMPLIFICATION: PP16
BI-210 STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS
BI-301 CODIS SAMPLE RECEIPT AND STIMAS ENTRY

Original protocols are archived with Laboratory Quality Manager.

New Protocols Instituted:

BI-105 O-TOLIDINE TEST FOR BLOOD
BI-302 CODIS SAMPLE DATA ENTRY AND UPLOAD
BI-303 CODIS DATABASE HIT VERIFICATION

Table of Contents

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2.0 ORGANIZATION AND MANAGEMENT

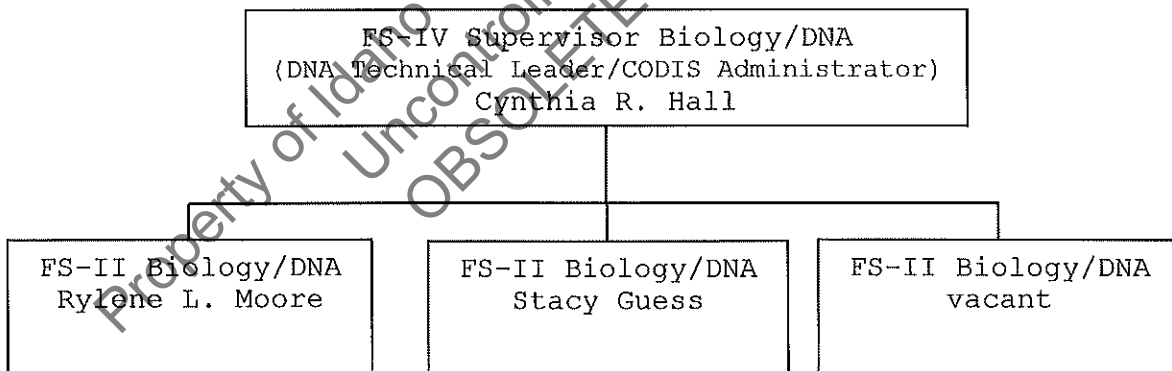
2.1 Organizational Chart and Functional Structure

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.



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

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:

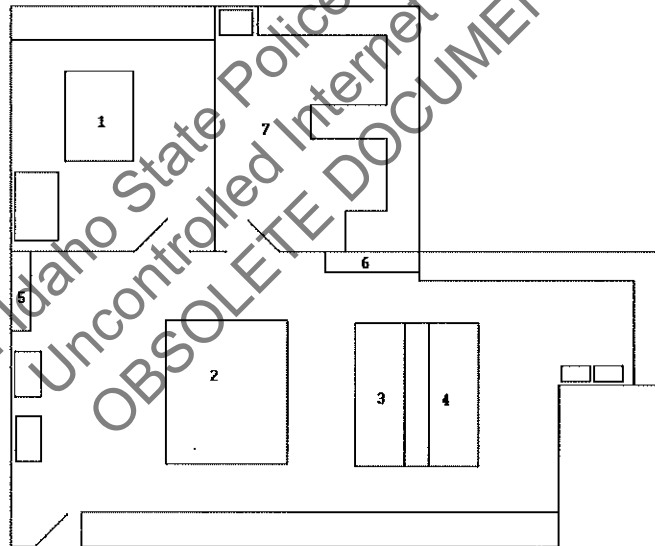
- 4.1.2.1 Only the CODIS State Administrator, designated Forensic Biology staff and CJIS personnel will have access to the CODIS Server.
- 4.1.2.2 A differential backup of the CODIS server will be performed each weekday. A full backup will be performed once weekly with the backup tape being stored off-site. 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.

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.



Biology Lab Areas

1. Evidence Screening/ALS
2. Evidence Screening
3. Analyst Workbench/DNA Extraction & Prep
4. Analyst Workbench/DNA Extraction & Prep
5. Chemical Fume Hood
6. Biological Hood
7. Amplification/ Post-Amp Room

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.

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.

3.3.3 DNA Analyst

The following delineate requirements for a DNA casework or database analyst whose responsibilities include performing genetic analyses on the 310 capillary electrophoresis instruments and data interpretation. DNA extraction and amplification set-up may be performed by appropriately trained laboratory technicians and/or those performing the biological screening of evidence following task-specific training and successful completion of a qualifying examination.

3.3.3.1 Education

Must have a Bachelor of Science in a biological science and successfully completed coursework in genetics, biochemistry, molecular biology and statistics.

3.3.3.2 Training

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

3.3.3.3 Experience

Must have a minimum of six months Forensic DNA laboratory experience.

3.3.3.4 Continuing Education

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

3.3.4 Forensic Biologist

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

3.3.4.1 Education

Must have a Bachelor of Science in a biological science.

3.3.4.2 Training

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

3.3.4.3 Experience

Prior to participating in independent 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.

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 cases submitted for analysis to Forensic Biology, in which reference sample(s) are present, and/or positive Biological screening results are obtained (See BI-102)

5.2.2 Limited Sample

In every case, care should be taken to save ~1/2 of a sample for independent testing. If testing would consume all or nearly all of a sample **and** there is an identified suspect charged in the case, the accused must receive appropriate notification. Notification will consist of a letter sent to the prosecuting attorney informing him/her of possible consumption and requesting the letter be forwarded to defense counsel. This letter will contain a date that testing will commence and allow for either: 1) testing by another accredited laboratory or 2) witnessing (by an acceptable expert) of the sample processing through the set-up of the genetic analysis run. An acceptable expert is a **scientist** with **'hands on', forensic experience** in both the technology used in the analyses, and the corresponding data interpretation. The notification letter will be sent a minimum of 14 days prior to the commencement of testing.

5.2.3 Amplified Product

Amplified DNA product will not be retained after 1) the report has been issued in the case or 2) review of the offender sample data has been completed and certified for CODIS entry.

6.0 VALIDATION

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

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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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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

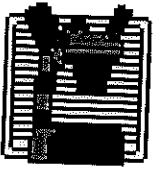
- BI-100** PROCESSING LIQUID BLOOD
- BI-102** DNA PACKETS
- BI-104** PHENOLPHTHALEIN TEST FOR BLOOD
- BI-105** O-TOLIDINE TEST FOR BLOOD
- BI-106** HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST
- BI-108** SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION
- BI-110** BIOLOGICAL SCREENING: 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

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)

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
420-QC QC STR KITS
422-QC 310 INJECTION LOG
426-QC ANNUAL NIST QC RUN

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MBI-100	
16/11/03	6-13-03
CRA	PIA

EXAMINATION OF BLOODSTAINED EVIDENCE

1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

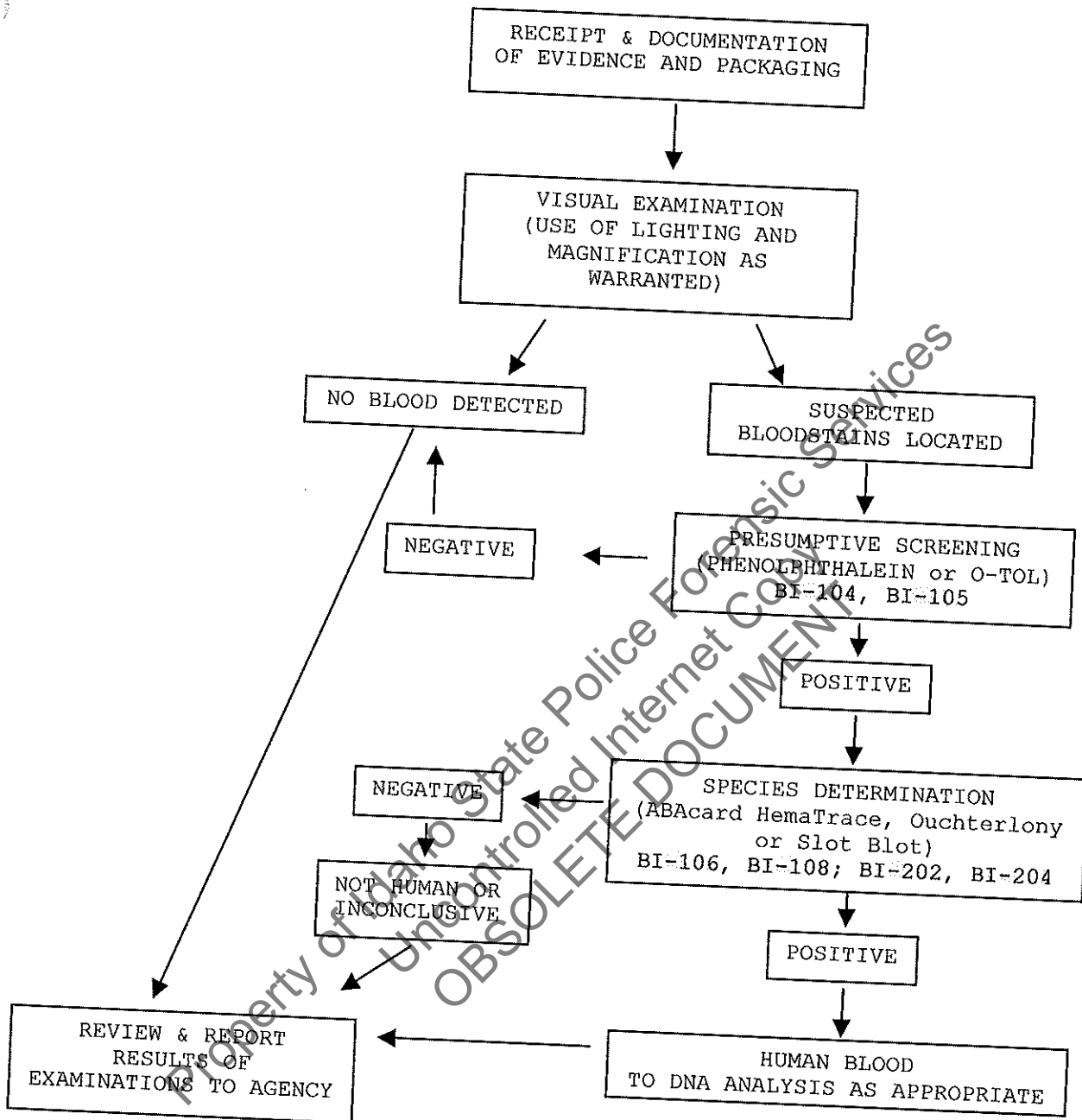
To provide uniform processing of evidentiary material for the presence of blood.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

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

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



MBI-102	
2/4/2003	2-4-2003
[Signature]	[Signature]

EXAMINATION OF EVIDENCE FOR SEMEN

1.0 BACKGROUND:

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

See Flow Chart on following page.



MBI-104	
L/11/03	6-13-03
CRA	RW

EXAMINATION OF EVIDENCE FOR BODY FLUIDS

1.0 BACKGROUND:

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

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

2.0 SCOPE:

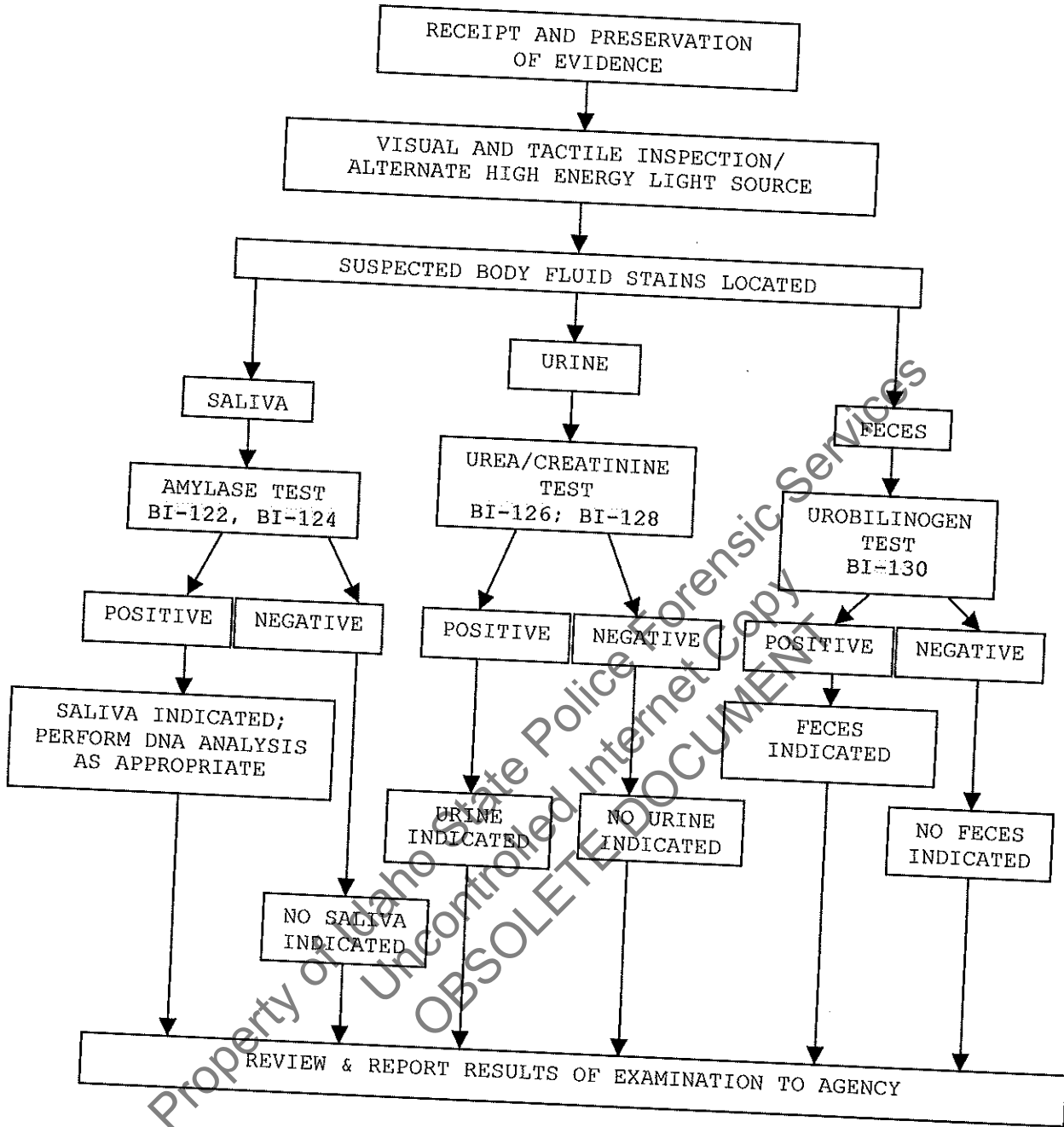
To provide uniform processing of evidentiary material for the presence of saliva, urine or feces.

3.0 EQUIPMENT/REAGENTS:

Normal room lighting conditions and the use of an 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:

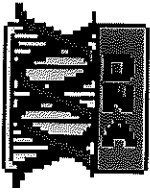
See Flow Chart on following page.



5.0 COMMENTS:

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

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



MBI-200	
L6/11/03	6-13-03
CRN	RJA

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.

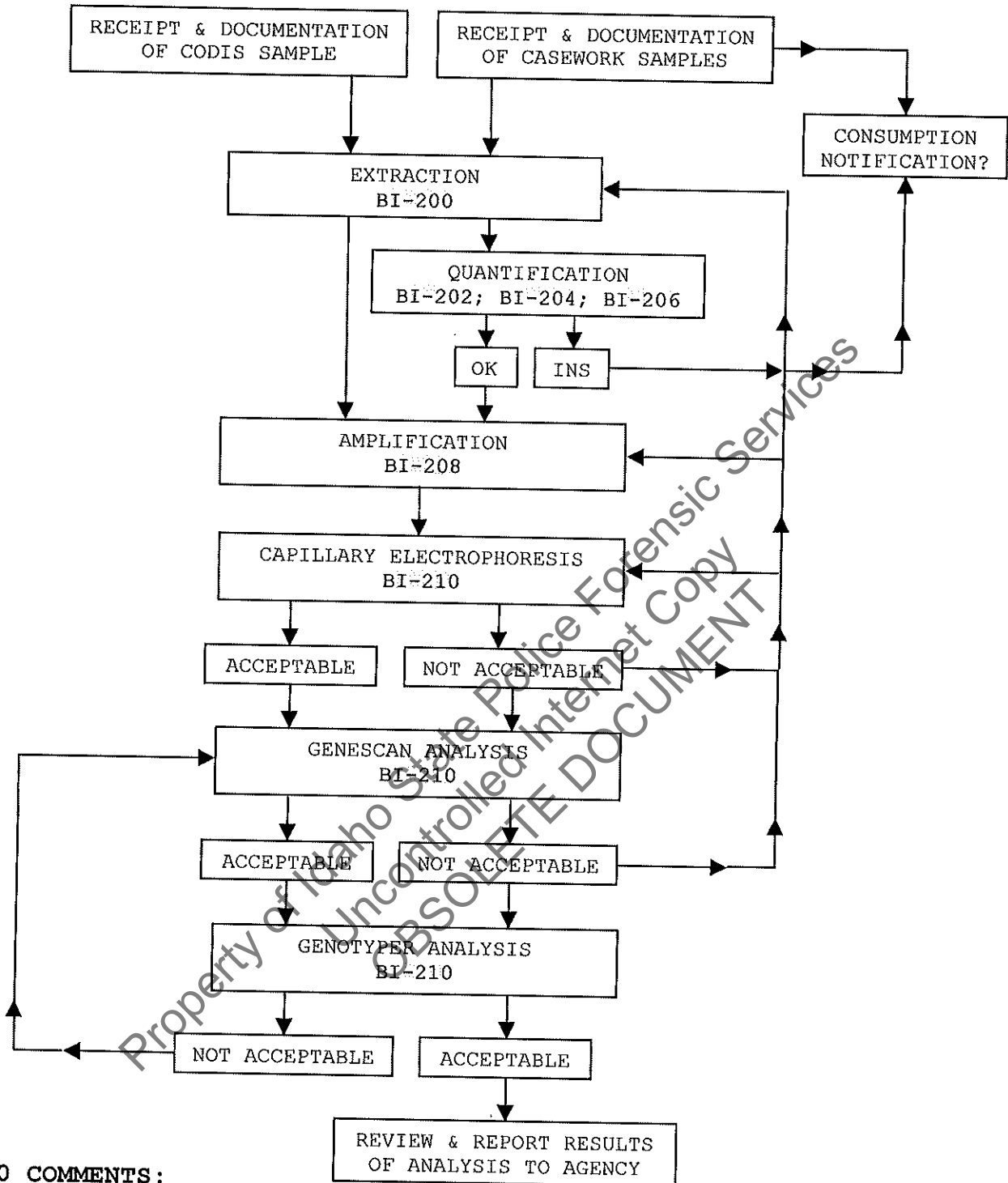
3.0 EQUIPMENT/REAGENTS:

As listed in individual analytical procedures.

4.0 PROCEDURE:

See Flow Chart on following page.

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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).



MBI-400	
6/11/03	6-13-03
CRN	RJG

DOCUMENTATION IN FORENSIC BIOLOGY

1.0 BACKGROUND:

Meticulous documentation, particularly for case notes and quality functions is an important aspect of forensic work. Careful reagent and equipment documentation ensures the easiest and most efficient mechanism for tracking problem sources and performing troubleshooting operations. In casework, the scientist's knowledge of case circumstance (and therefore their ability to discern potential significance) may be limited. It is also common to be called upon to testify months, or even years, after processing evidence for a given case. Careful observation and detailed note-taking will not only refresh the scientist's memory and provide support for the conclusion in the laboratory report, but might also provide additional information not thought to have been important at the time of evidence processing.

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 CHEMICALS/REAGENTS

4.1.1 COMMERCIALY 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 annually, at a minimum, 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.

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.

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)

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 (as known or appropriate): property number, description, location, serial number, estimated life expectancy, 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
Water Bath(s) Temperature Check
Heating Block Temperature Check
Oven Temperature Check

MONTHLY (Form 406-QC)

Pipettes Cleaned
Centrifuges Cleaned
BioSafety Hood(s) Flow Check and Clean
Eye Wash Station Check
pH Meter High/Low pH Check
PCR Lab Cleaned
Autoclave Clean and Check
Artel PCS2 Calibration Check

QUARTERLY

Thermalcycler Verification Tests (Form 408A-QC)
Balance Calibration Check (Form 408B-QC)
Chemical Shower Check (Form 408B-QC)
Centrifuge RPM Check (Form 408B-QC)

SEMIANNUALLY

Matrix for the ABI PRISM™ 310 Genetic Analyzers (The matrix used may or may not be replaced; best will be used). Data for each new matrix will be filed in the 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

In addition to the above schedule, personnel should check appropriate parameter function, on all instrumentation, with each use and perform MacIntosh HD Optimization as needed. Any problems noted should be brought to the attention of the necessary supervisory personnel and documented on Form 402-QC (the optimization will also be recorded on this form when it is performed).

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 Whenever feasible, every attempt should be made to gain entry into the evidence without breaking the original seals. Any seal altered or created by a scientist will bear their initials and date across the seal.
- 4.3.1.6 Evidence descriptions should be "unique" inasmuch as possible (i.e., one pair blue jeans is **NOT** adequate). They should include, as appropriate and necessary for identification, colors, sizes (measurements where appropriate- e.g., knife and blade), manufacturer, model, brand, serial numbers or other identifiers and condition (e.g., worn, clean, torn, mud-caked, blood-soaked, etc.).

4.3.1.7 Photography, digital or otherwise, is often useful in documenting the appearance of evidence items. However, it is not meant to completely replace drawing, but instead as a supplement or in cases when drawing may be too difficult to accurately depict the item. Careful drawing and description result in careful and detailed examinations and, in many instances, may be a better choice than photography.

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

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

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

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

An item with multiple sub-items

(e.g., a SAECK; Item 1)

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

4.3.1.9 The Biology Screen Case Summary Form (Form 101-BI) may be used for summarizing analyses.

4.3.1.10 If a form is used for more than one case, a copy of the 'completed' form should be made for any additional case files. Each copy should contain a reference regarding the location (case file) of the original document. For each file, the associated case should be listed and case data highlighted. In general, subfolders should be organized from front to back as follows: report, chronological case notes/forms, copy of chain of custody, phone/info log ('tangerine' paper may be used for ease of

identification), followed by agency materials submitted with evidence. When report has been issued, this documentation should be bound (e.g., stapled) together.

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

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

Signature

Name of Scientist
Title of Scientist

4.3.2.3 The following statements are to be used in a biology screening report, as dictated by the analysis findings (where appropriate, descriptions, quantity, and/or locations of individual stains may be included in the corresponding statements).

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 (or not performed).

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

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

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

"Preface see above" were not examined.

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

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

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

The following samples (or the samples listed below) 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 blood sample(s) from [list name(s)].

For reports in cases where sample quantity may be insufficient to obtain DNA results:

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

Evidence Disposition Statement:

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

Evidence Description Examples:

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

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

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

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

Deoxyribonucleic Acid (DNA) Analysis, employing the Polymerase Chain Reaction (PCR), 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 1.6×10^{10}):

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.)" ¹.

For statements regarding profile matches that do not meet the 'source attribution' criterion (estimated frequency in population of greater than 1 in 1.6×10^{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 of the population groups calculated) 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 description (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.

Or: (two person mixture example)

The DNA profile from "item description (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 description (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:

¹This 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 of the population groups calculated) frequency estimate", 2) a statistical frequency exceeding the source attribution criterion (for $N=1.6 \times 10^7$, $\alpha=0.01$; *Forensic Science Communications* 2(3) July 2000, and 3) that "name" does not have a genetically identical twin.

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

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

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

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

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

4.3.3 REVIEW

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

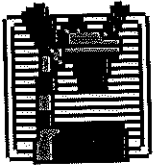
4.3.3.2 "Peer-review" in Forensic Biology will encompass both technical and administrative reviews.

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

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

4.3.3.5 The second scientist performing the review will initial each page (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.



BI-100	
6/11/03	6-13-03
CRN	RW

PROCESSING LIQUID BLOOD

1.0 BACKGROUND:

Most known reference standards in forensic casework are received in the form of liquid blood, generally in a lavender top tube. In a liquid state, these samples are more susceptible to degradation resulting in the loss of DNA. Bloodstains stored in a dry state, even at room temperature, may be suitable for DNA testing for many years.

2.0 SCOPE:

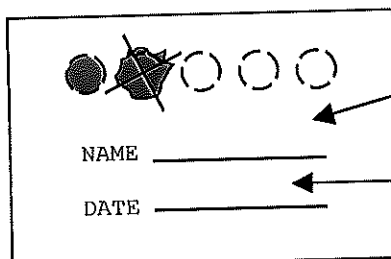
To provide a method for the creation of stable DNA samples from blood.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

- 4.1 Label stain card with a minimum of Case Number and Item Number, Date and Initials.
- 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).



Name and/or Case Number and Item Number
(e.g., John Doe/Doe, John or M20011234
It. 1A)

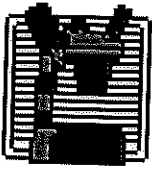
Date and Preparer's Initials (case number
and item number may also be placed here if
subject name is on line above)

- 4.4 Allow bloodstain card to air-dry completely before packaging.
- 4.5 Place dried stain card into coin envelope (~3½" 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 minimally.
- 4.6 Make Case DNA Packet (See BI-102) and place bloodstain sample inside.

5.0 COMMENTS:

- 5.1 Exercise caution and wear appropriate protective gear when preparing bloodstains (e.g., gloves, labcoat, protective eyewear).
- 5.2 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.

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BI-102	
LD/11/03	6-13-03
CRN	RJS

DNA PACKETS

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

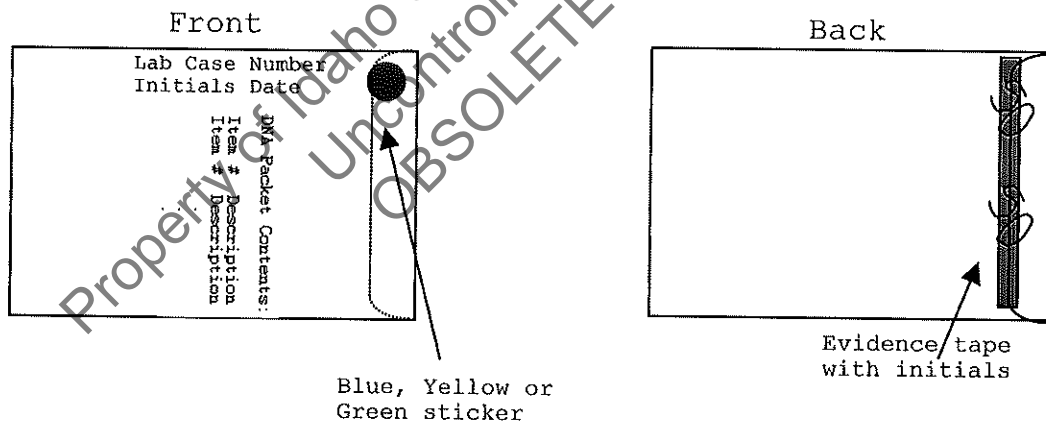
Schleicher & Schuell: Blood Collect Card(s) #903
Coin Envelopes (~3 $\frac{1}{8}$ " x 5 $\frac{1}{2}$ " and other sizes as needed)
DNA Packet Envelope (~6 $\frac{1}{2}$ " x 9 $\frac{1}{4}$ " manila envelope)

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. Swabs packaged in separate envelopes within an outer container (sexual assault evidence collection kits, for example) do not need to be repackaged into a new coin envelope. Each envelope will be labeled with Case Number, Item Number, Date, Scientist's Initials and sealed with evidence tape.

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

- 4.3 The DNA packet itself need not be sealed until biological screening of the case is completed and all samples are believed to have been collected.
- 4.4 DNA Packets for crimes without a statute of limitations (i.e., Homicides, and Sexual Assaults where DNA evidence exists 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 to allow for tracking in the ETS. The storage location will have a barcode.
- 4.6 DNA Packets will be stored at $\leq -20^{\circ}\text{C}$ as space allows, and then, if necessary, either returned to the submitting agency, or placed in room temperature storage after any requested DNA analyses have been performed. However, prior to return to a submitting agency, the Biology/DNA Supervisor should be notified to ensure maintenance on site is no longer necessary.

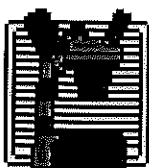


- 4.7 Following DNA testing, any leftover DNA extracts will be put into a plastic ziplock bag or coin envelope and placed in the DNA Packet. Individual tubes may also be sealed with parafilm or other sealant to prevent leakage and/or evaporation if desired.

5.0 COMMENTS:

- 5.1 The DNA Packet is NOT meant to contain "items of evidence" but rather biological samples that have been removed from items of evidence. Not every item or every stain on every item should be included in a DNA Packet. The person performing the biological screening should use discretion and prioritize sample collection contacting a DNA Analyst or the Biology Program Manager if necessary.
- 5.2 Given the small sample necessary for DNA testing, discretion should be used in determining the size of the stain cutting. Rarely, if ever, should a cutting exceed the dimensions of the coin envelope.
- 5.3 On **RARE** occasions when it is deemed necessary to have more stains collected in a given case than will fit into a single DNA Packet Envelope, the DNA Packets should still be entered into EIS as a single evidence item but labeled "1 of X, 2 of X ...X of X".

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BI-104	
LD/11/03	G-BOR
CRN	RDS

PHENOLPHTHALEIN TEST FOR BLOOD

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Phenolphthalein Stock
Ethanol
3% Hydrogen Peroxide
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

4.1 Positive (known bloodstain) and negative (sterile/nanopure H₂O) control samples are processed, prior to testing any forensic samples, to ensure the working stock reagents are functioning properly

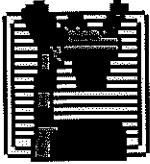
4.2 Cotton swabs or a folded piece of filter paper are used to collect the suspected blood onto the tip. A swab may be moistened with sterile/nanopure H₂O if necessary.

- 4.3 To the swab or filter paper 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% H₂O₂ 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 negative (-). 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% H₂O₂ are generally considered inconclusive.

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BI-105	
10/11/03	6-13-03
CRN	RDE

O-TOLIDINE TEST FOR BLOOD

1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 Positive (known bloodstain) and negative (sterile/nanopure H₂O) control samples are processed, prior to testing any forensic samples, to ensure the working stock reagents are functioning properly.

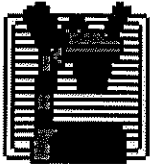
4.2 Cotton swabs or a folded piece of filter paper are used to collect the suspected blood onto the tip. A swab may be moistened with sterile/nanopure H₂O if necessary.

- 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% H₂O₂ 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 negative (-). 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% H₂O₂ are generally considered inconclusive.
- 5.3 O-tolidine is designated as a potential carcinogen and should be used with caution.

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BI-106	
L6/11/03	6-13-03
CRW	P.D.C.

HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABACard® Hematrace® Test Kit

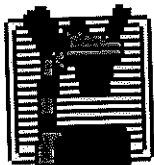
4.0 PROCEDURE:

- 4.1 Label extraction tubes for identification.
- 4.2 Using the buffer provided, allow samples (generally ~2mm x 2mm stain cutting) to extract at room temperature for 5-30 minutes (longer, if necessary for aged stains).
- 4.3 Label an ABACard® Hematrace® test device for each sample, including controls.
- 4.4 Apply ~150µl (4 drops with provided dropper) of a sample extract to the 'S' well of its corresponding test device and incubate at room temperature for ≤ 10 minutes.

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

5.0 COMMENTS:

- 5.1 Samples must be at room temperature for the test. If extracts have been stored in refrigerator/freezer, allow them to reach room temperature before proceeding.
- 5.2 Both positive (known human bloodstain) and negative (extraction buffer alone) controls are used.
- 5.3 Since the reaction time is dependent on hemoglobin concentration, as well as other sample-specific factors, it is necessary to wait the full 10-minute incubation before reporting a negative result. However, a positive reaction may occur in much less time.
- 5.4 As with any antigen-antibody reaction, false negatives (as the result of a "high dose hook" effect) may be produced with concentrated samples. When negative results are obtained with very 'heavy' stains, the sample should be further diluted and the test repeated.
- 5.5 Other reagents may be used for extraction. For example, 3-5% Ammonia Hydroxide (aged stains), saline, 1XPBS or PCR-TE. The volume used for extraction may be reduced for sample conservation or dilute stains (e.g., 150µl).
- 5.6 Although most nonhuman species tested do not produce a positive result with the ABACard® Hematrace® test, some crossreactivity has been reported (i.e., other primates, weasel, ferret, skunk). Therefore, 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, in many cases, by reducing the size of the sample to be extracted (e.g., a single 2mm thread).



BI-108	
LD/11/03	6-13-03
CRA	RMS

SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

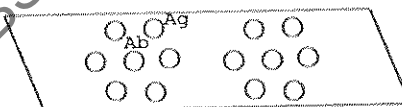
3.0 EQUIPMENT/REAGENTS:

- 3% Ammonium Hydroxide (for aged stains)
- Antisera
- Agarose, E25
- 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 dH₂O.
- 4.2 Extract a small sample (e.g., 2mm² bloodstain; 1cm² stain of other suspected body fluids) in ~100 µl dH₂O (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 dH₂O-moistened paper towel or filter paper).

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 (may be left overnight) to remove uncomplexed proteins.

4.7.2 Rinse the gel in dH₂O for ~5 minutes; dampen two pieces of filter paper with dH₂O and place on top of gel, followed by a stack of paper towels to serve as a wick. Place a weight on top of the paper towels to 'press' the gel for ≥ 30 minutes. Remove the weight, paper towels, and filter paper and dry the gel in an oven at 56°C-65°C for ≥ 20 minutes.

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

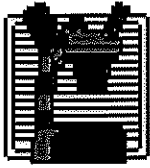
4.7.4 Destain until background is clear and blue precipitate bands can easily be seen.

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.



BI-110	
8/28/01	8/30/01
<i>[Signature]</i>	<i>[Signature]</i>

BIOLOGICAL SCREENING: USE OF ALTERNATE LIGHT SOURCE (ALS)

1.0 BACKGROUND:

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

2.0 SCOPE:

To provide a method for enhancing visualization/localization of physiological and trace evidence on evidentiary items.

3.0 EQUIPMENT/REAGENTS:

Alternate lighting Source
Filtered Safety Goggles

4.0 PROCEDURE:

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

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

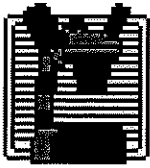
4.2 Follow manufacturer's operating instructions for specific details on equipment operation.

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

5.0 COMMENTS:

5.1 Failure to use safety goggles, or use of incorrect goggles could result in permanent eye damage. Read any manufacturer's safety guidelines provided with the equipment.

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BI-112	
Lo/11/03	6-13-03
CRV	RW

BCIP TEST FOR ACID PHOSPHATASE

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

BCIP Reagent
Oven or Water Bath
Cotton Swabs (optional: Filter Paper)
Test Tubes

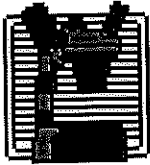
4.0 PROCEDURE:

- 4.1 Label test tubes for suspected semen stains and controls.
- 4.2 Lightly rub a known semen stain with a pre-moistened cotton swab and place swab in appropriately labeled test tube.

- 4.3 Repeat step 4.2 for suspected semen stains (or apply 10-20 μ l extract where appropriate) and use a pre-moistened swab for a negative control.
- 4.4 Add sufficient BCIP reagent to each test tube to cover the cotton tip.
- 4.5 Incubate for 20 minutes at 37°C.
- 4.6 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.



BI-114	
10/11/03	6-13-08
CRN	RDK

BRENTAMINE TEST FOR ACID PHOSPHATASE

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Brentamine Solution A
Brentamine Solution B
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

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

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

- 4.3 Lightly rub a suspected semen stain with a pre-moistened cotton swab, or press a moistened piece of filter paper against the stain.
- 4.4 Add Brentamine Working stock to sample(s) and observe for the appearance or absence of a pink to purple color change.
- 4.5 To avoid false positives, the results should be recorded as positive(+), as indicated by the development of the above color change, or negative(-) 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 Positive reactions, though generally weak, may be obtained on anal/rectal and some vaginal swabs in absence of any semen.
- 5.2 Test may also be performed using 10-20 μ l of a sample extract.
- 5.3 This test may also be used for mapping large, possible semen stains via a moistened paper transfer method. A sheet(s) of moistened filter paper is pressed against the item of evidence. Marks are made on the paper to indicate the edges of the evidence for orientation of any subsequent color reaction. The paper is sprayed with Brentamine Reagent and analyzed as above.
- 5.4 Fast Blue B is a possible carcinogen and should be handled cautiously



BI-116	
10/11/03	6-B03
CRN	RDS

SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

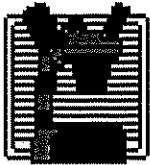
4.0 PROCEDURE:

- 4.1 Label tubes with identifying information.
- 4.2 Take a sample (~3-5m² portion of stain or ~1/8 each of one or two cotton swabs), transfer to the appropriately labeled tube and extract in a minimal volume (50µl - 100µl) of dH₂O at RT for ≥ 20 minutes.
- 4.3 At this point, agitation, vortexing, brief sonication and/or piggyback centrifugation may be used to assist in removing sperm/cellular material from the substrate.
- 4.4 Mix/resuspend the sample for use in microscopic examination (BI-118) and/or p30 detection (BI-120). Alternatively, the supernatant may be removed, without disturbing the pellet, for additional testing [e.g. AP screening (BI-112; BI-114), p30, etc.] prior to resuspension.

5.0 COMMENTS:

- 5.1 Other reagents may be substituted for dH₂O (e.g., 1XPBS, PCR-TE, saline) in 4.2.
- 5.2 The sample sizes and extraction volumes are those typically used and are recommendations. The scientist has the discretion to increase or decrease the sample size and corresponding extraction volume as case circumstances dictate.
- 5.3 While the primary use of this liquid extract is for semen identification testing, these extracts may be used for other screening tests as well (e.g., saliva, urine, feces).
- 5.4 The sample may optionally be extracted in dH₂O directly on the microscope slide at the analyst's discretion. However, the quantity of sperm observed may be diminished and no sample will remain for further testing (e.g. p30) when using this method.

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BI-118	
10/11/03	6-13-03
CRA	RDC

SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION

1.0 BACKGROUND:

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

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

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

4.0 PROCEDURE:

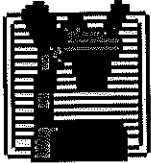
- 4.3 Cover the heat-fixed sample extract with Xmas Tree Stain Solution A and allow staining for ≥ 15 minutes at RT.
- 4.4 Remove the stain with a gentle stream of dH₂O and cover the stained area briefly (~15-20 seconds) with Xmas Tree Stain Solution B. Remove this stain with a stream of EtOH (95% or Absolute).
- 4.5 Allow the slide to dry and apply mounting medium or dH₂O and a cover-slip prior to microscopic examination.
- 4.6 Scan the slide on $\geq 200X$ magnification. Sperm heads will retain the red stain, while the tails, if present, will appear green. Use 400X magnification if necessary to verify sperm morphology.
- 4.7 Documentation in notes should include the following:
 - 4.7.1 A description of the condition of the sperm seen (e.g., heads only; mostly heads, some intact etc.,).
 - 4.7.2 An estimate of the number of sperm seen per field (e.g., 12/slide; 0-1/200X; 3-5/200X; 5-10/200X; $>10/200X$; or 1+ - 4+ etc.).
 - 4.7.3 The presence of any epithelial cells (e-cell) and their number (e.g., rare, occasional, few, moderate, many, or 1+ - 4+). The scientist may also note e-cell descriptions [e.g. nucleated (NEC or nuc.) or anucleated (ANEC or Anuc.)] and whether or not there are large squamous epithelial cells present.
 - 4.7.4 If the situation arises in which there are only one or two sperm heads, a single intact sperm, or a few sperm heads of questionable morphology, a second qualified scientist must 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.

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

5.0 COMMENTS:

5.1 Stains purchased commercially have expiration dates, 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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BI-120	
16/11/03	6-13-03
CPA	RDS

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

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABACard® p30 Test Kit

4.0 PROCEDURE:

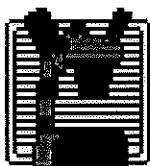
4.1 Label an ABACard® p30 test device for each sample, including controls.

4.2 Add 10µl of each sample, to include both positive (known semen stain extract or Seri™ semen standard [10ng; 10µl of a 1:100 dilution]) and negative (saline) controls (see BI-116), to ~190-200µl (4 drops) of saline and mix thoroughly.

- 4.3 Transfer each extract (~200µl) to the 'S' well of the appropriately labeled test device and incubate at RT for 10 minutes.
- 4.4 A positive result is indicated by the appearance (within 10 minutes) of a pink line in both the control 'C' and test 'T' areas. A negative result is indicated by the absence of a pink line (after 10 minutes) in the 'T' area of a test device. Results are inconclusive anytime a pink line fails to develop in the 'C' area.

5.0 COMMENTS:

- 5.1 Samples must be at room temperature for the test.
- 5.2 Other reagents may be substituted for saline (e.g., 1XPBS, PCR-TE, dH₂O) in 4.2.
- 5.3 Since the reaction time is dependent on p30 concentration, as well as other sample-specific factors, it is necessary to wait the full 10-minute incubation before reporting a negative result. However, a positive reaction may occur in much less time.
- 5.4 As with any antigen/antibody interaction, excess antigen may lead to a 'high dose hook' effect resulting in false negatives when the p30 concentration is very high. This effect should be considered when examination and presumptive tests have indicated the likelihood of the presence of semen. In those instances, the sample should be diluted and the test repeated.



BI-122	
16/11/03	6-13-03
CRW	RDE

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.

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

Phadebas Test
Page 1 of 2

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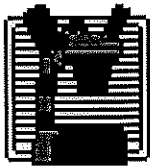
BI-122
Revision 1
06/2003

4.0 PROCEDURE:

- 4.1 Stain samples (~2-5mm²; 1/8-1/4 swab; 20µl extract) and controls [20µl dH₂O is used for negative control; 20µl of 1:100 and 1:500 dilutions of fresh saliva and either neat saliva, or a saliva stain (≤2mm² cutting) as positive controls] are placed into appropriately labeled tubes.
- 4.2 Add 1ml dH₂O 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 If the blue color of a sample is as dark or darker than that of the 1:100 control, it is an indication of an elevated level of amylase and is reported as 'detected (or indicated) 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.



BI-124	
10/11/03	6-13-03
CEA	RPS

AMYLASE TEST (STARCH IODIDE)

1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

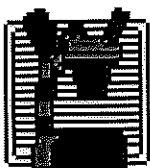
4.0 PROCEDURE:

4.1 Sample and control extracts (dH₂O is used for negative control) should be prepared in dH₂O 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 overflow) 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 dH₂O), let stand a few moments to develop the purple color, then pour it off the plate's surface.
- 4.7 Record the results by measuring the diameter of the clear circles. For reporting, see 5.1.

5.0 COMMENTS:

- 5.1 Positive controls should include 1:100 and 1:500 dilutions of fresh saliva as well as neat saliva or an extract of a known saliva stain. If the clear zone of an extract \geq 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.



BI-126	
8/28/01	10-3-01

DETECTION OF URINE (UREASE)

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Urease Reagent
Small Corks (to fit 12x75mm test tubes)
12x75mm test tubes
Red Litmus Paper

4.0 PROCEDURE:

- 4.1 Cut out ~2.0cm² piece of suspected urine stain and controls, cut them into small pieces and place them into appropriately labeled 12x75mm test tubes.
- 4.2 Add 3-4 drops of dH₂O and 6-7 drops of Urease Reagent to each test tube.
- 4.3 Cut a slit into the bottom of each cork and insert a small piece of red litmus paper into the slit.

4.3 Place a cork (with litmus paper) into each tube; do not allow the litmus paper to come into contact with the liquid.

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

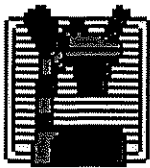
4.5 Note and document any change in the color of the litmus paper that occurs within the incubation time. A positive reaction (+) is recorded when the red litmus paper turns blue. When there is no color change noted, a negative (-) result is recorded.

5.0 COMMENTS:

5.1 Controls include positive (known urine stain) and negative (dH₂O blank) and a substrate control where appropriate and available.

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

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BI-128	
8/28/01	10-3-01
	R128

DETECTION OF URINE (CREATININE)

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

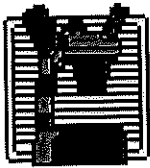
4.1 Cut out ~0.5cm² piece of suspected urine stain and controls and place them into appropriately labeled 12x75mm test tubes.

4.2 Add 0.5ml of dH₂O to each test tube and extract for 15 minutes at RT.

- 4.3 Remove the substrate. Add 1 drop (~50µl) of Picric Acid Solution and 1 drop (~50µl) of 5% NaOH to each tube.
- 4.5 An orange color develops fully within 15 minutes and is stable for approximately 2 hours. The orange color is a positive indication of Creatinine. The negative control stain solution should remain yellow.
- 4.6 Document results in case notes. Record positive (+) or negatives (-). Analysts may use other descriptive word(s) (e.g., strong, weak,) or numerical grading (e.g., 1+ - 4+) as well.

5.0 COMMENTS:

- 5.1 Controls include positive (know urine stain) and negative (dH₂O blank) and a substrate control where appropriate and available.
- 5.2 This method is not specific for Creatinine. Although other chromagens are detected by this procedure, their concentrations are negligible.
- 5.3 Among other substances, glucose is reported to produce an orange color with alkaline picrate, although the color is pale. However, if there is likely to be confusion between this and a urine stain, the addition of 2 drops of glacial acetic acid renders a creatinine-containing sample pale yellow after a few minutes. (The color can be restored by adding a few drops of 5% NaOH). Heat is necessary to achieve the color change to pale yellow if the stain is glucose.
- 5.3 The Creatinine Test is one of many presumptive tests for urine; a confirmatory test for the identification of urine in a dried stain is not available.



BI-130	
8/28/01	10-3-01
	N/A

DETECTION OF FECAL MATERIAL (UROBILINOGEN)

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

10% (w/v) Mercuric Chloride Solution
10% (w/v) Zinc Chloride Solution
Amyl (Isopentyl) Alcohol
12x75mm test tubes

4.0 PROCEDURE:

4.1 Cut out ~0.5cm² piece of suspected fecal stain and controls and place them into appropriately labeled 12x75mm test tubes.

4.2 Extract samples in ~3 drops of dH₂O for 15-30 minutes at RT.

- 4.3 Remove the material and add ~3 drops of 10% Zinc Chloride Solution to the extract.
- 4.4 Add 5 drops of Amyl Alcohol to the extract and vortex.
- 4.5 Spin sample for 5 minutes on low (~2000 rpm) in the serological centrifuge and transfer the upper phase to a new 12x75mm tube.
- 4.6 To the upper phase, add 3 drops of 10% Mercuric Chloride Solution and observe any color change under both white and long wave UV light.
- 4.7 A positive reaction is recorded when green fluorescence is visible under long wave UV light. Absence of green fluorescence under long wave UV light is recorded as a negative reaction. Under white light, the solution may become rose-pink if urobilin is present.

5.0 COMMENTS:

- 5.1 Controls include positive (known fecal stain) and negative (dH₂O blank) and a substrate control where appropriate and available.
- 5.2 The Edelman's Urobilinogen Test is one of many presumptive tests for feces; there are no confirmatory tests available for the identification of fecal material.
- 5.3 The production of a green fluorescent complex is indicative of feces from humans and other carnivores. Due to the presence of chlorophyll, the feces of herbivores will produce an orange-pink fluorescence in this test.



BI-200	
10/11/03	6-13-03
CRA	PDC

EXTRACTION PROTOCOLS FOR PCR DNA TYPING TESTS

1.0 BACKGROUND:

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

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT:

Centricon® Concentrator 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 should be set up first.

Caution: See Comments 1 and 4.

5.1 BLOODSTAINS/SALIVA SAMPLES (OTHER NON-SEMEN SAMPLES):

5.1.1 For stains on cloth or porous materials, Add an ~3mm² cutting/sample to a sterile 1.5ml tube (for smaller stains consume sample). For stains deposited on non-porous objects, collect sample using a cotton-tipped applicator with a small amount of sterile deionized water, TE or SEB. Cut a swab portion to yield an equivalent stain amount (~3mm²) 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.

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 Centricon concentration (See 6.0). A second addition of PCIAA may be added to the aqueous layer, if necessary (e.g. large or 'dirty' interface), before proceeding.

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 H₂O
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 all but ~50µl of the supernatant, taking care not to disrupt the cell pellet in the bottom of the tube. Transfer this supernatant (♀/epithelial cell fraction) to 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, may not be sufficient to remove all of the ♀ contribution. In addition to the sperm cell washes that follow, the initial extraction (5.2.1-5.2.2) may, at the scientist's discretion, be repeated 1-2 times at this point. The incubation time may also be increased (up to overnight for a single incubation). This may be particularly useful in instances in which there is an overwhelming proportion of epithelial cells with the presence of sperm that appear intact microscopically. Following the final incubation, the extract should be transferred to a new sterile 1.5ml tube and spun as in 5.2.4. These additional supernatants do not need to be retained.
- 5.2.7 Wash the sperm pellet as follows: Resuspend the pellet in 500µl of Sperm Wash Buffer (SWB). 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, fewer washes with repeated ♀ extraction incubations may be performed. Alternatively, the volume of SWB may be increased to 1 ml for each of the washes.

5.2.9 To the remaining sperm pellet solution add:

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

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

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

Note: At this point, DNA isolation/clean-up may be performed using either ethanol precipitation or Centricon concentration (See 6.0). A second addition of PCIAA may be added to the aqueous layer, if necessary (e.g. large or 'dirty' interface), before proceeding.

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 may be washed to reduce surface dirt and contaminants. This may be accomplished by immersing the hair(s) in sterile, deionized water in a sterile, 50 ml conical tube. Each hair to be analyzed should be washed separately in fresh water. Alternatively, the hair(s) may be placed in a 1.5ml tube containing 1ml 10% SDS and sonicated briefly. Again, each hair to be analyzed should be treated separately.

5.3.3 Even if the hair(s) was washed prior to proceeding to 5.3.4, it may still have cellular material on its surface that did not originate from the hair donor. Therefore, in addition to cutting off ~0.5 - 1.0cm of the root-end, a 0.5 - 1.0cm cutting of the shaft adjacent to the root 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, containing the hair sample, Add:

500µl SEB
20µl 1M DTT
10µl ProK

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 **Optional** If the hair has not completely dissolved at this point, the analyst may add:

20µl 1M DTT
10µl ProK

Incubate at 56°C for 26 hours. Vortex 30 seconds. The sample may now be spun for 1 minute at high speed at room temperature to remove pigment and particles. Transfer the supernatant to a new sterile 1.5ml tube.

5.3.6 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 Centricon concentration (See 6.0). A second addition of PCIAA may be added to the aqueous layer, if necessary (e.g. large or 'dirty' interface), before proceeding.

5.4 EXTRACTION FROM SALIVA (ENVELOPE FLAPS AND STAMPS) :

- 5.4.1 Cut an $\sim 1.0\text{cm}^2$ 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).
- 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:

400 μl SEB
10 μl ProK

Incubate overnight at 56°C .

- 5.4.6 In a fume hood, add 500 μl 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 Centricon concentration (See 6.0). A second addition of PCIAA may be added to the aqueous layer, if necessary (e.g. large or 'dirty' interface), before proceeding.

5.5 EXTRACTION OF DNA FROM SALIVA (CIGARETTE BUTTS) :

- 5.5.1 Remove $\sim 5\text{mm}^2$ section of the paper near the butt end of the cigarette, in an area likely to have had contact with the smoker's mouth. Cut the paper into smaller pieces, if necessary, and put them into a sterile 1.5ml tube.

5.5.2 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).

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

5.5.4 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 Centricon concentration (See 6.0). A second addition of PCIAA may be added to the aqueous layer, if necessary (e.g. large or 'dirty' interface), before proceeding.

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 ml's of ether in a 15-ml polypropylene tube).

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

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

5.6.4 Crush bone/tooth into small pieces with mortar and pestle or blender (chisel, hammer and press may be used initially). Note: This is best performed using a pre-chilled mortar and pestle. Place the pre-chilled mortar on a bed of dry ice (in a styrofoam box) to keep the evaporation of liquid nitrogen to a minimum. Add a small amount of liqN₂ and crush to as fine a powder as possible, adding more liqN₂ 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 liqN₂, tilt mortar toward pouring spout and slowly transfer to a 50-ml polypropylene tube. Allow the liqN₂ to evaporate completely.

5.6.5 To the tube, add:

400µl SEB
10µl ProK

Mix thoroughly by hand and transfer extract (using a 1ml pipette, with tip end cut-off, or large bore transfer pipette) to a 1.5ml microcentrifuge tube and incubate at 56°C overnight.

5.6.6 In a fume hood, add 500µl PCIAA to the extract. Mix vigorously by hand to achieve a milky emulsion. Spin in a microcentrifuge for 2-3 minutes to achieve layer separation. Transfer aqueous layer to new sterile tube. Repeat this step until the interface is clean.

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

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

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

5.7.5 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 (>10,000 rpm) for ~15 minutes. Gently decant supernatant. Continue to transfer additional 1.5 ml of cold sample to the same tube, centrifuge, and discard supernatant until the entire sample has been consolidated.

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

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

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

5.8 EXTRACTION FROM BLOOD/SALIVA ON FTA CARDS:

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

- 5.8.1 Remove a "punch" from the FTA card using a 1.2mm Harris punch (this is accomplished by placing punch firmly on card and twisting 1/2 turn clockwise and 1/2 turn counterclockwise). Eject sample(s) into microAmp tube(s).
- 5.8.2 Add 150µl FTA reagent to microAmp tube(s), mix and incubate at RT for ~5 minutes.
- 5.8.3 Remove and discard FTA reagent from sample(s) (using either vacuum with small pipette tip or by micropipette).
- 5.8.4 Repeat 5.8.2-5.8.3 twice.
- 5.8.5 Add 150µl TE to microamp tube(s), mix and incubate at RT for ~5 minutes.
- 5.8.6 Remove and discard TE from sample(s) (using either vacuum with small pipette tip or by micropipette).
- 5.8.7 Repeat 5.8.5-5.8.6 twice.
- 5.8.8 Make sure the punch is at the bottom of the microAmp tube(s), using a sterile pipette tip if necessary. Place tubes, uncovered in 65°C oven for ≥2 hours.
- 5.8.9 Proceed to PCR Amplification (see BI-208).

6.0 DNA ISOLATION PROCEDURES:

Two methods are available for isolating DNA, ethanol precipitation and using a Centricon concentrator device. It is the scientists' decision which method to use. Centricon concentration 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 25 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; however, larger volumes may be used for highly concentrated samples) at 56°C for a minimum of 30 minutes.
- 6.1.7** Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

6.2 ISOLATION VIA CENTRICON CONCENTRATOR 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 ~20-40µl.

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

7.0 DNA SAMPLE CLEAN-UP:

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

7.1 MICROCONCENTRATOR:

7.1.1 Assemble Microcon-100 according to manufacturers instructions.

7.1.2 Prewet the filter with 50µl TE.

7.1.3 Pipette the sample into the upper reservoir.

7.1.4 Fill remaining volume with TE.

- 7.1.5 Spin in a fixed rotor centrifuge at 2500 rpm for 20 minutes.
- 7.1.6 Discard the effluent in the lower reservoir as needed.
- 7.1.7 Repeat steps 7.1.4-7.1.6 two more times.
- 7.1.8 Remove the filter unit and invert it into a new tube.
OPTIONAL: 10µl of PCR TE can be added to the filter and vortexed briefly before inverting the filter unit into a new tube.
- 7.1.9 Spin in a fixed rotor centrifuge at 500-2000 rpm for 2-5 minutes to collect sample. The final volume of retentate should be about 30-50µl.
- 7.1.10 Estimate the amount of DNA in the extracts using slot blot hybridization (see BI-202; BI-204).

7.2 CHELEX CLEAN-UP:

7.2.1 To the DNA sample(s) (~40-50µl), add 200µl freshly prepared 5% Chelex.

Option: Add 10µl Pro K.

Note: This procedure may also be used for direct extraction of 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 taken directly to slot blot hybridization (see BI-202; BI-204) for quantification of the DNA at this point, or, optionally, cleaned-up using a microconcentrator device [see 7.1; the procedure (7.1.7) does not need to be repeated in this instance] prior to quantification.

note: care must be taken to not disturb the Chelex resin when removing sample for subsequent procedures.

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 ($\sim 100\mu\text{l}$) of differential extraction buffer (see 5.2.1) may be added directly to the slide. Incubate 15 minutes at RT and then by pipetting up and down, wash the sample off of the slide and transfer to 1.5ml tube. Repeat 3-4 times and proceed to 5.2.1.

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

8.2 SOAKING IN XYLENE:

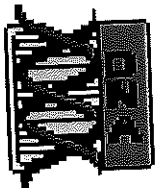
- 8.2.1 Soak the slide in xylene for several hours until the cover slip can be slid or pried from the slide. **Note:** This will likely remove markings from the slide.
- 8.2.2 Remove the hair and soak in about 10-20mls xylene to remove the residual mounting medium.
- 8.2.3 Rinse the hair briefly in absolute ethanol to remove xylene and proceed to hair extraction under 5.3.

9.0 Comments:

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

- 9.2 An appropriate reagent blank (for each type of extraction) should be carried through all extraction steps to check the purity of the reagents being used. There need only be one reagent blank per extraction run, it is not necessary to have a separate one for each case that is extracted at the same time.
- 9.3 Presoaking bloodstains with PBS may help to prevent inhibition of amplification by heme products, particularly when analyzing DNA obtained from samples of "heavy" bloodstains (e.g. control bloodstains). Additionally, using microconcentrator devices for DNA isolation may be more effective in removing heme and other inhibitors than ethanol precipitation.
- 9.4 These extraction methods employ the use of liquid nitrogen and dry ice. Both of these substances may cause severe burns. Double-glove or wear "cold" gloves while performing these procedures and exercise caution.
- 9.5 These procedures represent the 'usual' protocol for a given material, however, any of these different extraction methods are suitable for all biological materials, though minor modifications may be necessary.

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BI-202	
20/11/05	6-15-03
CRA	P. De

DNA QUANTIFICATION: QUANTIBLOT

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 40 μ l to the 'A' tube.
- 4.1.3 Dispense 20 μ l of PCR-TE into tubes B-H.
- 4.1.4 Prepare a serial dilution series by mixing and subsequent 20 μ l transfers from tubes A through G. H will contain PCR-TE only and serve as a negative control. Store standards at -20 $^{\circ}$ C between uses. The dilution series consists of 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng in 5 μ l, respectively. Another suitable standard may be substituted, using a similar dilution series.

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.
- 4.2.3 Prepare the DNA standards, and samples by combining 5 μ l with 150 μ l of spotting solution (w/o stain). 1.25ng of previously quantified 9947A may be used as a 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.

4.2.6 Once all samples are in wells, slowly turn on the sample vacuum for ~30 seconds or until all samples have been drawn completely onto the membrane. Release the clamp vacuum, disassemble the slot-blot apparatus, remove the membrane and proceed to 4.3.

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% H₂O₂. 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 and 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 and 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.

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 an 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.

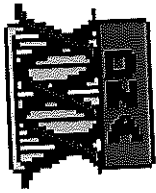
5.0 COMMENTS:

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 Mannheim 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.



BI-204	
8/28/01	W/d/c
J	R/A

DNA QUANTIFICATION: ACES

1.0 BACKGROUND:

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

"Simple protocols for typing forensic biological evidence: chemiluminescent detection for human DNA quantitation and restriction fragment length polymorphism (RFLP) analyses and manual typing of polymerase chain reaction (PCR) amplified polymorphisms." Budowle, B. et al. *Electrophoresis*, Vol. 16, No. 9, 1995, pp.1559-67.

"A chemiluminescence-based detection system for human DNA quantitation and restriction fragment length polymorphism (RFLP) analysis." Guisti, AM; Budowle B. *Appl Theor Electrophor*, Vol. 5, No. 2, pp. 89-98.

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

"ACES™ Human DNA Quantitation System Instruction Manual." Gibco BRL Life Technologies, Inc.

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
Denaturation/Spotting Solution (DS)
Neutralization Solution (NS)
ACES™ Final Wash Solution
Chemiluminescence Reagent

Hybridization Container
Kodak Image Station
ACES™ Kit Components
Nylon Membrane
2X SSC
PCR-TE
UV Crosslinker
Oven

4.0 PROCEDURE:

4.1 IMMOBILIZATION OF DNA

- 4.1.1 Fill out the slot-blot form (Form 206-BI). The ACES™ human DNA standards should be applied in order of decreasing concentrations (e.g., 4.0 ng/μl, 2.0 ng/μl, 1.0 ng/μl, 0.4 ng/μl, 0.2 ng/μl, 0.1 ng/μl, 0.04 ng/μl, 0.02 ng/μl, 0.01 ng/μl, 0.004 ng/μl, and the ØDNA control). The remaining 37 slots are available for various samples and controls.
- 4.1.2 Cut a piece of neutral nylon membrane (e.g., Biodyne® A; ~7.5 x 11.5 cm.) marking a corner for orientation by cutting it off. Pre-wet the membrane in a sufficient volume (completely covering membrane) of 2X SSC and incubate on shaker/rotator for approximately 5 minutes at room temperature.
- 4.1.3 Combine DNA samples (10 μl of each ACES™ standard, and an appropriate volume of each sample based on expected yield - ~5-10 μl) with 400 μl of Denaturation/Spotting Solution (0.5M NaOH, 0.5M NaCl) in designated wells of a 96-well microtiter plate. Use 1.0 ng previously quantified 9947A DNA as a control.
- 4.1.4 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.
- 4.1.5 Carefully pipet samples into appropriate slot-blot wells, avoiding bubbles and contact with the membrane.
- 4.1.6 Once all samples are in wells, slowly turn on the sample vacuum allowing them to be drawn onto membrane for ~30 seconds.
- 4.1.7 Leaving the sample vacuum on, rinse each well with an additional 400 μl (2 x 200 μl) of Denaturation/Spotting Solution and allow it to be pulled slowly through the membrane.

- 4.1.8 After all of the buffer has been pulled through the membrane, release the clamp vacuum, remove the membrane from the slot blot apparatus and place it in a sufficient volume of Neutralization Solution (0.2M Tris-HCl pH 7.5, 2X SSC). Rinse at room temperature on shaker/rotator for approximately 5 minutes.
- 4.1.9 Remove the membrane from the Neutralization Solution and lightly blot it between pieces of 3MM-type filter paper. Transfer membrane to a new filter paper sandwich and bake it in an oven at approximately 80°C for ~30 minutes. (Alternately, the membrane can be allowed to air dry prior to crosslinking.)
- 4.1.10 Check performance of X-linker before each use. This is done by pressing 'Intensity' followed by start. If 'bulb' flashes they need to be replaced. Place the dried membrane, DNA side up, into the X-linker. Press 'Optimal X-link' and then 'Start'. Remove membrane and proceed with hybridization.

4.2 DNA HYBRIDIZATION

4.2.1 Make a 1:5 dilution of the ACES™ Wash Buffer I Concentrate (you will need approximately 200mls, i.e., 40mls wash: 160mls dH₂O). Pre-warm the diluted wash buffer, ACES™ Hybridization solution (you will need approximately 15mls), and a shaking H₂O bath to 50°C. Make a 1:10 dilution of the ACES™ 10X Final Wash Buffer and equilibrate at room temperature (you will need approximately 100mls). **Note:** Dilutions are made with nanopure H₂O.

4.2.2 Place the membrane in a tray (e.g., Hybriboat™) containing 10ml of pre-warmed ACES™ Hybridization solution. Optional: Cover solution with a clear Hybriboat™ plastic membrane, being careful to avoid any large air bubbles. However, if not using plastic membrane, double volume of hybridization solution (20ml). Cover tray and pre-hybridize for ~20 minutes at 50°C in a shaking water bath.

4.2.3 Pour out the pre-hybridization solution and add 5ml fresh hybridization solution containing 2.5µl of the D17Z1 DNA probe. Replace the Hybriboat™ plastic membrane (or use 10ml hybridization buffer) and cover as above; hybridize for ~20 minutes at 50°C in a shaking water bath.

4.2.4 Following hybridization, pour off hybridization solution and briefly rinse membrane 2X in a small volume of the pre-warmed ACES™ Wash Buffer I (1:5). Replace Hybriboat™ lid, as before, and wash membrane 2X for ~10 minutes each at 50°C in a shaking water bath, using ~1/2 of the remaining Wash Buffer for each wash (~90mls each). The Hybriboat™ plastic membrane is not needed with these larger volumes.

4.2.5 Pour off the wash solution and add ~50 ml of ACES™ Final Wash Buffer (1:10). Wash membrane 2X for ~ 5 minutes each at room temperature on shaker/rotator.

4.3 DETECTION

4.3.1 After final wash, drain as much of the wash buffer as possible from the hybridized membrane (draining onto paper towel or blotting residual buffer in tray with a Kimwipe, if necessary).

4.3.2 Using a sterile pipet, add 5ml of chemiluminescence reagent (e.g., Lumi-Phos® Plus or CDP-Star®) to the hybridized membrane. Cover and rotate by hand for ~5 minutes, ensuring uniform coverage of the membrane. A Hybriboat™ plastic membrane may be used to ensure complete membrane coverage (or add 10ml of the chemiluminescence reagent). Alternatively, the chemiluminescence reagent (5ml) may be placed directly on the Image Station platen and the membrane placed (DNA side down) on top of it.

4.3.3 Using a forceps, remove the hybridized membrane from the tray and allow the chemiluminescence reagent to drain off. Touch the end of the membrane to a paper towel or filter paper to remove any residual liquid and proceed with membrane processing on the Kodak Image Station (BI-206).

5.0 COMMENTS:

5.1 DO NOT allow the membrane to dry out at any time during this procedure.

5.2 Thoroughly clean the slot blot apparatus (e.g., Neutrad).

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BI-206	
LD/11/03	6-13-03
CEA	RDS

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").

- 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; this may also be performed as a single exposure of varying times, based on the analysts' training and experience) 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, or instead of using min and max.

4.2 BAND LOCALIZATION AND QUANTIFICATION:

- 4.2.1 On the vertical tool bar at left, click on . 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.

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:

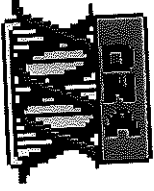
4.3.1 Use the 'ROI' to view the image only and use File: Print. This brings up the Print box on the computer screen. Check the for Image, and deselect any other boxes. Go to 'image layout' to see how it will look, then select 'OK'. In the printer screen, click 'Setup', 'Properties', 'Graphics' and click 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.

5.0 COMMENTS:

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



BI-208	
10/11/03	6-13-03
CPA	M D L

STR AMPLIFICATION: PP16

1.0 BACKGROUND:

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

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

GenePrint® PowerPlex™ 16 System Technical Manual

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

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

4.1.2 The amount of DNA template added to an amplification reaction should be targeted at 0.5-1.0ng for 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 Determine the number of samples to be amplified and label microAmp tubes (200μl) for identification. Label a microfuge tube(s) for the Master Mix. Place the labeled sample tubes in a rack or microAmp tray. The scientist may choose to irradiate the tubes with UV light at this point (≥ 15 minutes) while performing other preparations.

4.2.2 Thaw the Gold STAR 10X Buffer and the PowerPlex™ 16 10X Primer Pair Mix.

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

Gold STAR 10X Buffer	2.5 μ l
PowerPlex™ 16 Primer Mix	2.5 μ l
*AmpliTaq Gold®	0.8 μ l
¹ DNA Template + dH ₂ O	19.2 μ l

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 50U/ μ l. Check tube to verify concentration and adjust volume as necessary to add 4U of enzyme per reaction.

¹For FTA samples (primarily offender samples) there is no volume for the DNA template so 19.2 μ l of dH₂O will be added to these tubes.

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

4.2.5 If DNA concentrations were not the same, add appropriate volume of dH₂O as necessary.

4.2.6 Pipet each DNA sample into the appropriate tube. Only the tube to which the DNA is being added should be opened at this time and only one DNA-containing tube should be open at any time (with the exception of the negative control which remains open throughout the process). Use 9947A control DNA for the positive amplification control and dH₂O for the negative amplification control. Again, making additions to the negative control last.

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

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

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

4.3 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 (typically FTA/offender samples) use 'pp16buccal'; the cycling conditions are as follows:

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

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

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

60°C for 45 minutes, then:

4°C soak

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

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

60°C for 45 minutes, then:

4°C soak

If, from the data generated on the 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. See BI-210 4.4.2.5 RFU Threshold for additional information.

4.4 AMPLIFIED DNA PRODUCT:

4.4.1 After cycling has concluded remove samples from thermalcycler. Samples should be run on the 310 Genetic Analyzer as soon as possible after amplification. Prior to 310 run and/or before analysis is completed the samples may be stored at 4°C. For longer storage periods, samples should be frozen at -20°C. Amplified product is **ONLY** 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 disposed of in a biohazard container in the amp/post-amp room. As needed, this container will be sealed and 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.
- 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 STAR 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).



BI-210	
10/11/03	0-1303
CPA	RDE

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, TH01, 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 1×10^{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.

GenePrint® PowerPlex™ 16 System Technical Manual

ABI PRISM™ 310 Genetic Analyzer User's Manual

Genescan® Analysis Software User's Manual

Genotyper® Software User's Manual

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 AMPLIFIED FRAGMENT DETECTION USING THE 310

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

4.1.1 Turn on instrument, turn on computer and refer to ABI PRISM® 310 Genetic Analyzer's User's Manual for detailed instructions on instrument set-up. Shut down is performed in opposite order (computer, then 310). The computer may be shutdown after each run; the 310 should only be shut down if it will not be in use for extended periods. Fill-in appropriate information in the 310 Injection Log (Form 422-0C).

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, 'CCD DUMMY' and 'SEQFILL DUMMY' may be added (generally, as the last two samples). There is a 'dummy' sample sheet already on the instrument so that these samples may be placed in a pre-run by themselves, rather than adding them to the new sample sheet. Enter appropriate identifying information for other samples into the sample column as follows:

Matrix samples: FLUOR, JOE, TMR or CXR

Allelic Ladder: LADDER (or PP16 LADDER)

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

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

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

4.1.4 Select a run module with the following settings:

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

Inj.Secs:

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.

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.

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). Using chooser file-sharing, copy the run folder and sample sheet to an analysis computer. After analysis and review are complete, a copy of the analyzed run folder for each case will be stored on an 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 contain the laboratory case number, as they will be separate projects. Open the project, or 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, minimally.

4.2.3 Set the parameters for Genescan® Analysis:

For Matrix Generation, review the Raw Data of the Fluor, JOE, TMR and CXR standards and record an 'x' value that is after the primer peak, in an area of relatively flat baseline signal for each matrix standard. Note the 'Analysis Range' must include ≥ 5 peaks for each matrix standard.

Use FILE\NEW\MATRIX to bring up a window to select the analysis parameters. Click on a 'dye color' and select the corresponding Matrix Standard (e.g., Blue dye =FLUOR standard), and then enter the 'x' start value that you recorded from the Raw Data for that sample. Repeat for each of the Matrix Standards and click 'OK' and a new Matrix file will be generated.

Check to see that the numerical value trends indicate a good matrix (numbers on diagonal are '1.0000' and decrease from that value in each column). Use FILE\SAVE AS\ to save new Matrix file (name is letter for instrument [M or R] followed by "MATRIX" and then the date "MMDDYYYY").

Check Matrix quality by applying it to the matrix samples. Select those samples in the Analysis Control Window and, using Project\Install\New Matrix, apply Matrix and analyze the data. Examine data in the Results Control Window. The samples should have peaks in the standard color but profiles should be relatively flat in the other 3 colors. With the exception of TMR (yellow) into CXR (red), bleed-through should not exceed 10%. If satisfied, save a copy of the Matrix to the ABI Folder in the System Folder (make color correction matrices if necessary). Print out Matrix Table and a 4-color electropherogram plot, from the Results Control Window, for each Matrix Standard. File in QC log for appropriate instrument.

For standard runs, review the raw data for all of the project samples and controls to determine the start and stop points of the analysis range. Select points that will not include the primer peaks but will cover the size range of 80 to ≥ 500 bases. 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 50 rfu at the analyst's discretion and must be noted on the GT electropherogram. Lowering of rfu threshold below 70 rfu (to ≥ 50) should be done with caution and only if the data generally appears to be good, and without excessive baseline background or artifacts. Peaks below 50 rfu are deemed inconclusive.

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

Size Calling Method: Local Southern

Split Peak Correction: None

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.

4.2.4 GENESCAN® DATA EVALUATION

4.2.4.1 Review Raw Data in the Analysis Control window to identify baseline problems, off-scale data, excessive 'spikes' or other anomalies that may interfere with data analysis and require re-injection or other corrective measures.

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

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

- 4.2.4.4 All negative controls (including reagent blanks) should be examined to verify that each displays a relatively flat baseline in blue, green and yellow.
- 4.2.4.5 Review all samples and evaluate: peak height and shape, matrix quality, and individual sample profiles. It is a good time to determine if the rfu cut-off will need to be changed in the Analysis Parameters of certain samples. Rfus should ideally be \leq 4000. However, data may be acceptable to 8,000 rfus if the overall data (according to the evaluation above) is of good quality.
- 4.2.4.6 After analysis and review are complete, SAVE PROJECT AS: Laboratory Case Number (minimally) 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 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 until burned to a CD for archival purposes. Case-specific CDs will be made for discovery upon request.

4.3 ALLELE ASSIGNMENT: GENOTYPER®

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 ~70%) 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

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 be deemed inconclusive. Data may be deemed acceptable if all alleles are present (though some are below 150-rfu threshold) **AND** the other positive control (Blind Control) appears as expected (i.e. the problem is confined to the 9947A sample).

LOCUS	GENOTYPE	LOCUS	GENOTYPE
D3S1358	14,15	TH01	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 only in this control, the data for other samples associated with that amplification need not be deemed inconclusive. This occurrence should be documented and the scientist's determination (and basis for it) documented in the case file.

4.4.1.4 The purpose of a **BLIND CONTROL** sample is primarily to assess correct genotyping, however, it does take measure of all of the steps in the analytical process from extraction through allele designation. The blind controls are made in batches and given random numbers. The scientist is not aware of the genotype of the sample. A blind control 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.

4.4.2 RFU THRESHOLD:

- 4.4.2.1 For CODIS samples and reference blood or oral standards (excluding autopsy samples that may be degraded or of limited quantity), a minimum of 100 rfu should be achieved for data acceptance. If necessary, go back in the process as follows: repeat injection (changing injection time; 3-10 seconds allowable range), or perform re-analysis (i.e., changing amount of amplified product added for fragment analysis), or re-amplification (increase DNA template), or re-extraction.
- 4.4.2.2 For minor mixture components (or low-copy single-source forensic samples), a threshold of 50 rfus may be used (see 4.2.3 Peak Detection). However, depending on signal/baseline may be deemed inconclusive.
- 4.4.2.3 Peaks below 50 rfu may (based on data obtained) be reported for exclusionary purposes only.
- 4.4.2.4 Peaks >8000 rfus will not be interpreted; the sample must be diluted, re-injected (3-10 seconds), reanalyzed (decrease the amount of amplified product added) or re-amplified (decrease DNA template) as deemed appropriate by the scientist. Peaks between 4000-8000 rfu may be interpreted (see 4.2.4.5) or repeated at the scientist's discretion. Application of a color corrected (CC) matrix may be necessary for higher rfu samples.

4.4.2.5 The additional cycle (+3AMP) option may only be used 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 ($\geq 70\%$) are ≥ 50 rfu and appear relatively balanced within a locus. The 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 meet or exceed 100 rfus at all loci for CODIS samples and any loci used for statistical inclusion of forensic samples.

4.4.3 MIXTURES

- 4.4.3.1 If, after the elimination of possible stutter and/or bleed-through, a profile shows at least 3 peaks at 2 or more loci, this is strong evidence of a mixture.
- 4.4.3.2 Loci that 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".

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 $f_{\text{STR profile}} = 2 \times 10^{-14}$, $\text{RMP} = 1 \text{ in } 5 \times 10^{13}$; 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:

$$\text{LR} = \frac{P(\text{evidentiary STR profile} | H_1)}{P(\text{evidentiary STR profile} | H_2)}$$

The larger the **LR**, the more likely H_1 was the true hypothesis (See BI-400) for reporting of statistical frequencies). For a paternity calculation, this corresponds to the **PI** (Paternity Index).

The **PE** (P_E) represents the probability that a randomly selected individual would possess one or more alleles inconsistent with the crime scene stain (or paternity). It is the complement of the **RANDOM MAN NOT EXCLUDED** (or "inclusion probability"; P_I).

The **PE** does not take into account the number of contributors, the principals' genotypes (i.e., the fact that they could account for the profile) or the evidence (e.g., peak height differences allowing probable donor assignment). It is calculated as follows:

$$P_E = 1 - P_I$$

Where $P_I = (p_1 + p_2 + p_3 \dots p_x)^2$ (the square of the sum of the frequencies of all alleles present in the evidentiary sample).

The $P_{E\text{combined}}$ (for all of the loci combined) is as follows:

$P_{E\text{combined}} = 1 - [(1 - P_{E1})(1 - P_{E2})(1 - P_{E3}) \dots (1 - P_{E15})]$
(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).

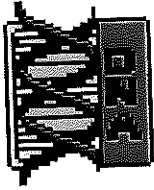
The equation $p_x \leq 1 - (1 - \alpha)^{1/N} \approx \alpha/N$, is used to determine maximum RMP (p_x) that would support 'source attribution' for a relevant population sample size (N) and selected confidence limit (i.e., $\alpha=0.01$; $1-\alpha=99\%$ confidence).

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 $>3.9 \times 10^{-11}$ (or less than 1 in 2.6×10^{10}) would confer 99% confidence that the evidentiary profile is unique in the population. However, an

additional 10-fold conservation factor, as recommended in NRC II, is added to this figure resulting in a frequency of less than 1 in 2.6×10^{11} for the reporting of source attribution.

In Idaho, using 2000 consensus figures, an operational population (N) of 1.6×10^7 has been selected (representing the sum of the populations of Idaho and the six surrounding states: ID= 1.3×10^6 ; MT= 0.9×10^6 ; NV= 2.0×10^6 ; OR= 3.4×10^6 ; UT= 2.2×10^6 ; WA= 5.9×10^6 and WY= 4.9×10^5). Therefore, an RMP of less than 1 in 1.6×10^{10} (including 10-fold conservation) will define source attribution (at 99% UCL) for analyses performed in this laboratory (See BI-400) for reporting of statistical frequencies).

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BI-301	
10/11/03	6-13-03
CRW	MAY

CODIS SAMPLE RECEIPT AND STiMAS ENTRY

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. It is important however, that samples entered into the database be given a unique identifier, which does not include any personal or identifying information, in order to maintain the confidentiality of the individual. Each laboratory must develop a method of identifier assignment so that each sample may be tracked, and identified at a later time, if the need arises.

2.0 SCOPE:

To provide a method for tracking offender database samples submitted for STR testing and CODIS entry, while ensuring individual confidentiality.

3.0 EQUIPMENT/REAGENTS:

CODIS Computer Workstation
Barcode Equipment
Database Samples and Report Forms

4.0 PROCEDURE:

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 retained after STiMAS data entry.

4.2 STiMAS ENTRY (Pre-entry Search):

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).

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	
Ld/11/03	6-13-03
CRW	RDC

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

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 'read' must also be entered for the individual samples through 'PCR Analysis'. This may be done by the analyst entering the sample data, or by another analyst logging on and entering the allelic data in the second read box. A 'check' indicates agreement between readers at individual loci and discrepancies in entry must be rectified before transfer to LDIS. Once all readings are in agreement, the individual performing the second read will do the transfer.

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 is in LDIS, by either mechanism, it is available for upload to SDIS.

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.

5.0 COMMENTS:

- 5.1** Refer to CODIS Training Manual and course documentation for more specifics if necessary.
- 5.2** The CODIS software is redundant and there is generally more than one way to accomplish many tasks. Using a mechanism other than that listed here is acceptable.
- 5.3** The CODIS software is updated periodically and any necessary changes in procedure provided with new updates supercedes those in procedures written prior to update, if appropriate.



BI-303	
2/4/2003	2-11-03
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CODIS DATABASE HIT VERIFICATION

1.0 BACKGROUND:

The implementation of the Combined DNA Index System (CODIS) in forensic DNA laboratories has provided an additional tool in assisting law enforcement agencies in solving or linking crimes that otherwise may not have resulted in the identification of a suspect. This is accomplished by the electronic storage and maintenance of DNA profiles at the local, state and national levels. Hits are obtained when a candidate match(es) is identified through a database search at any level. Hit verification involves evaluating the candidate match to determine if it is a true match and verification of CODIS offender sample data where necessary and possible.

2.0 SCOPE:

To provide a method of sample verification to be performed prior to law enforcement agency notification of a database hit to ensure reporting of only true, confirmed matches.

3.0 EQUIPMENT/REAGENTS:

CODIS
NDIS Procedure Manual, CODIS Training Manual/documentation
STiMAS
AFIS
Offender Database Sample(s)
Equipment/Reagents for STR Analysis

4.0 PROCEDURE:

4.1 MATCH VERIFICATION (Forensic):

4.1.1 For 'hits'/matches involving an ISP Forensic Biology evidentiary sample (either case-to-case or case-to-offender) the primary responsibility for match verification follow-up lies with the Idaho CODIS Administrator.

4.1.2 The CODIS Administrator or designee will first evaluate the 'Candidate Match' in Match Manager to determine if there is a basis for exclusion and, therefore, disposition as 'No Match'. A 'No Match' disposition doesn't require further verification or, where applicable, notification of the other laboratory involved with the match. However, the CODIS Administrator or designee will make an effort to communicate this information to the CODIS Administrator of the other laboratory in cases of matching at moderate stringency or high stringency at several loci (where another laboratory is involved).

4.1.3 If evaluation in Match Manager demonstrates that the candidate match consists of potential high stringency (e.g., exclusions attributable to different typing systems, Promega v. Applied Biosystems), or possibly moderate stringency in the event of a forensic mixture or degraded sample, the disposition is changed from 'candidate Match' to 'Pending' until the verification process is complete. In general, for case-to-case matches, the verification will consist of communication between scientists regarding the data, while case-to-offender matches typically necessitate sample verification at the 'offender lab'. Once the status of the 'candidate match' has been resolved, the disposition is set accordingly (e.g., 'No Match', 'Offender Hit', 'Forensic Hit'). If verification results in a 'hit', a hit report form is completed and, along with the match report, is filed in the CODIS file (also a copy to the case file for forensic hits). The appropriate law enforcement agency is notified of the 'hit'. If the law enforcement agency submits a sample from the identified offender, appropriate analysis and issuance of a supplemental report will be performed as in 4.2.5.

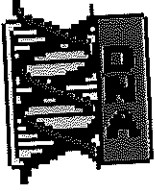
4.2 MATCH VERIFICATION (Offender):

- 4.2.1** For 'hits'/matches involving an ISP Forensic Biology convicted offender sample the primary responsibility for match verification follow-up lies with the CODIS Administrator for the laboratory with the forensic (evidentiary) sample. However, the initial evaluation in Match Manager, (see 4.1.2-3) and AFIS sample verification (see 4.2.2) will be initiated as soon as feasible. In addition, if more than five working days have passed since match identification, the Idaho CODIS administrator or designee will initiate contact with the other laboratory.
- 4.2.2** Once a potential match has been verified, The 'Browse Offenders' function in the STIMAS application will be used to search for the offender sample ID and the corresponding submission form will be printed. The associated offender sample folder will be retrieved from the secure file cabinet and taken to BCI for an AFIS search of the thumbprint to verify identification of the offender. All documentation will be filed in the CODIS file.
- 4.2.3** Following AFIS verification of the thumbprint, re-analysis of the offender sample will be performed as appropriate (i.e., if duplicate analysis has already been performed either as a QC function or as the result of an inadvertent duplicate, analysis will not be repeated) prior to agency notification.

4.2.4 Following sample verification (AFIS and re-analysis) the forensic case laboratory, in the case of an interstate hit, or submitting law enforcement agency will be notified of the confirmed hit. Laboratory notification may be made verbally and relevant documentation will be provided to the forensic case laboratory as requested. In Idaho, initial notification as well as the request for a new DNA sample from the identified offender, may be made verbally. However, written notification and a formal request for a new DNA sample, in the form of a report, will be sent to the appropriate law enforcement agency.

4.2.5 For intrastate offender hits (Idaho), where possible, a newly obtained DNA sample from the offender will be analyzed with all deliberate speed. The analysis of the forensic sample may also be repeated, though this is not required. A supplemental report will be issued delineating the match in the usual manner (i.e., same treatment as for matching suspect sample submitted with case evidence).

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BI-310	
8/28/01	10-3-01
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CODIS SAMPLE EXPUNGEMENT

1.0 BACKGROUND:

Participation in the National DNA database, in accordance with the DNA Analysis Backlog Elimination Act of 2000, necessitates provisions for DNA profile expungement in the event that a qualifying offender's conviction is overturned. Additionally, the Idaho DNA Database Act of 1996 addresses court-granted expungement requests (I.C. §19-5513). Removal of DNA profile data and/or destruction of biological samples obtained from Convicted Offenders may be necessary as a result of conviction reversal or sample collection/submission errors. Expungement is defined as the removal of DNA profile data from local (LDIS), state (SDIS) and national (NDIS) databases, removal of identifying information from other laboratory documentation and destruction of the biological sample from which the offender database DNA profile was generated.

2.0 SCOPE:

To provide a protocol for CODIS sample expungement that protects an individual's rights to privacy and maintains the integrity of the Idaho DNA database program. All procedures will be documented on the CODIS Sample Expungement Checklist (Form 310-BI).

3.0 EQUIPMENT/REAGENTS:

CODIS Worstation
STIMAS Database

4.0 PROCEDURE:

4.1 EXPUNGEMENT VERIFICATIONS

Prior to removal of any DNA profile data, source identification, or biological sample destruction, the CODIS Administrator or designee will verify: 1) the authenticity and validity of the request/order for expungement, 2) that the offender has no other qualifying offense(s) and 3) the identification of the original DNA database sample.

4.1.1 REQUEST/ORDER VERIFICATION

Prior to Request/Order verification, a search of the DNA STIMAS is performed to establish whether or not the specified sample is in the database. If the sample is not in the database, the submitting party will be contacted for resolution and the resulting action documented by written communication with the party. Expungement requests may be received in the form of a court order or an official letter from the Idaho Department of Correction or Idaho Attorneys' General Office. A written request from any other party will be referred to ISP legal staff and no action will be taken until legal has made a determination. Any questions or concerns regarding the validity of an expungement request will also be referred to ISP legal staff. Authenticity of the request/order document will be verified by contact with the submitting party.

4.1.2 RECORD VERIFICATION

To ensure that the offense for which the expungement is requested is the only qualifying offense, a request will be made of BCI to perform a criminal history check. If this is not the case, contact (both by phone and in writing) will be made with the submitting party to communicate why the request for expungement is denied. A copy of the expungement order and criminal history check will accompany the letter to the submitting party. If necessary, the request will be forwarded to ISP legal staff for resolution.

4.1.3 DNA SAMPLE VERIFICATION

The identity of the original database sample must be verified before any data are expunged or samples destroyed. Where possible, the original database sample will be reanalyzed to verify/identify the correct database profile. A new DNA sample from the offender must then be submitted to verify sample identification, ensuring removal of the correct sample.

4.2 EXPUNGEMENT

Once all of the verification steps have been accomplished (e.g., it has been determined that the offender sample is actually in the database, that it should be expunged, and that the correct sample has been identified), the following procedures will be performed by the CODIS Administrator.

4.2.1 The DNA Collection report, associated with the specified sample, will be located and marked as "EXPUNGED". This form will be initialed, dated, and, along with a copy of expungement request/order, returned to submitting party with an expungement notification letter. Since this form contains personal identification information, no copy of this record will be retained.

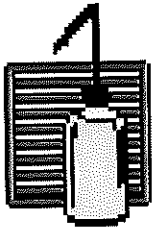
4.2.2. The sample will be removed from the STiMAS database using the 'sample expungement' function. BCI will be notified to change criminal history form of the offender to reflect that a DNA sample does not exist.

4.2.3 The data for the specified sample will be removed from existing databases (i.e., LDAS, LDIS and SDIS). After sample removal, a full upload to NDIS will be performed to remove sample at the national level. The sample deletion will be reported (in writing) to the NDIS Custodian and a deleted specimen report will be requested. A copy of all deleted specimen reports will be included with the expungement notification letter sent to the submitting party.

4.2.4 Both the original DNA sample and the verification DNA sample, that was submitted for expungement, will be destroyed in the presence of another scientist.

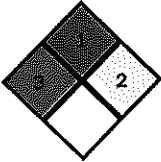
4.3 DOCUMENTATION

4.3.1 A copy of the expungement request/order will be made. The original expungement request/order, along with copies of all deletion reports and the completed CODIS Sample Expungement Checklist will be sealed and filed (by submitting party and date) with the laboratory QA/QC Manager. The original completed CODIS Sample Expungement Checklist along with copies of all specimen deletion reports will be sealed and filed (under offender number and date) with the CODIS Administrator. No documentation containing the offender's name or similar identifying information (including the expungement request/order) will be maintained in Biology. An expungement notification letter, along with the original DNA collection report (that has been marked "expunged") and copies of all documentation, will be sent to the submitting party.



Form 100-BI

PHENOLPHTHALEIN REAGENT



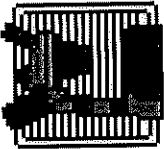
Phenolphthalein 2.0g
KOH 20.0g
Zinc (granular) 20.0g

Phenolphthalein, KOH, and 100ml of dH₂O are refluxed with Zinc until solution is colorless (producing phenolphthalin in ~1-3 hours). Store solution refrigerated in dark container to which ~5g fresh zinc has been added. Remove for stock dropper bottle as needed.

* May also be purchased commercially as phenolphthalin by itself or in 'kit form' with ethanol and 3% hydrogen peroxide for performance of the 3-stage presumptive test.

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Date	Initials	Pheno Source/Lot	KOH Source/Lot	Zinc Source/Lot	Lab Lot# Reagent Name
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO
					PHENO



Scientist: _____

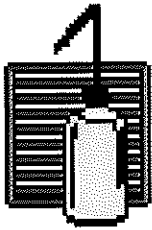
Case Number: _____

Date: _____

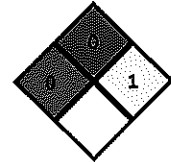
BIOLOGY SCREENING SUMMARY

SAMPLE ID	BLOOD		SEMEN	SALIVA	URINE	FECES
	Pheno	Hematrace				

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HYDROGEN PEROXIDE 3% (v/v)



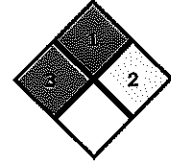
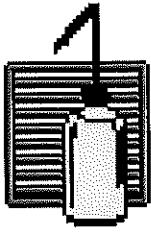
Generally a commercial purchase, however, may be made from a 30% Solution (which is a commercial purchase) as follows:

Hydrogen Peroxide (30%) 10ml/90ml nanopure dH₂O

Mix the H₂O₂ with 90ml of nanopure dH₂O and store at ~4°C.

Date	Initials	30% H ₂ O ₂ Source/Lot#	Lab Lot# Reagent Name
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
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			HP

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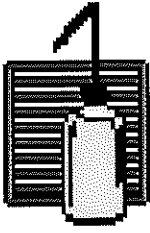
O-TOLIDINE REAGENT

O-Tolidine 0.6g
Glacial Acetic Acid 100ml
Ethanol 100ml

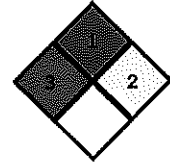
Dissolve O-tolidine in Acetic Acid/Ethanol mixture consistent with ratios above. O-tolidine is light sensitive and should be stored in dark reagent bottle and kept refrigerated when not in use.

Date	Initials	O-tolidine Source/Lot	Acetic Acid Source/Lot#	Ethanol Source/Lot	Lab Lot# Reagent Name
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL
					O-TOL

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Form 104-BI



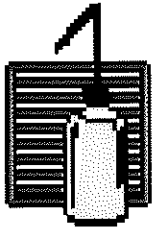
AMMONIUM HYDROXIDE (~3%)

Ammonium Hydroxide (Concentrated ~30%)

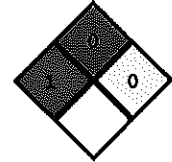
10ml/100ml

Add the NH₄OH to 90ml of nanopure dH₂O, mix well and store at RT.

Date	Initials	NH ₄ OH Source/Lot#	Lab Lot# Reagent Name
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH



Form 106-BI



SODIUM CHLORIDE (NaCl) 1M

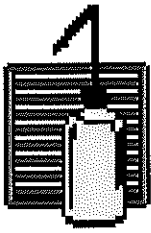
NaCl 28.2g/500ml

Dissolve the NaCl in 500 ml nanopure dH₂O, autoclave and store at RT.

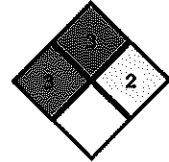
or

Make a 1:5 dilution of 5M NaCl (Form 233-BI) in nanopure dH₂O, autoclave and store at RT.

Date	Initials	NaCl Source/Lot#	Lab Lot# Reagent Name
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl
			1NaCl



Form 108-BI

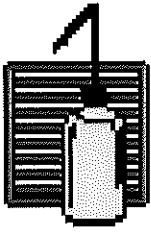


OUCHTERLONY DESTAIN

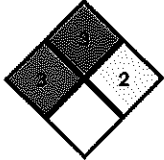
Methanol 50ml
Distilled water 50ml
Glacial Acetic Acid 10ml

Date	Initials	MeOH Source/Lot#	Acetic Acid Source/Lot#	Lab Lot# Reagent Name
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD
				OD

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Form 110-BI



OUCHTERLONY STAIN

Ouchterlony Destain (Form 108-BI) 44ml

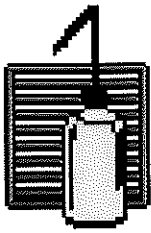
Add one of the following to Destain. Mix well and store at RT.

Naphthalene Black 10B (Amido Black; 0.1%) 44mg

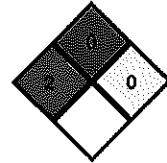
or

Coomassie Blue (Brilliant Blue R; 0.2%) 88mg

Date	Initials	Destain Lab Lot#	Stain Source/Lot#	Lab Lot# Reagent Name
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS
				OS



Form 112-BI



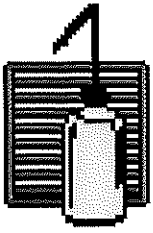
BCIP REAGENT

Sodium Acetate Trihydrate 68 mg
BCIP (5-Bromo-4-Chloro-3-Indolyl Phosphate) Disodium Salt 25 mg

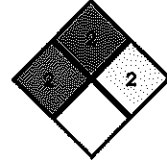
Dissolve the Sodium Acetate Trihydrate in 50 ml nanopure dH₂O.
Dissolve the BCIP in the Sodium Acetate Trihydrate solution.
Refrigerate in a dark container.

Date	Initials	Sodium Acetate Trihydrate Source/Lot#	BCIP Source/Lot#	Lab Lot# Reagent Name
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP
				BCIP

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Form 114-BI



10X BRENTAMINE (SODIUM ACETATE) BUFFER

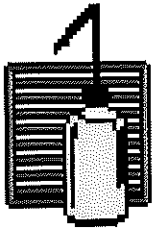
Sodium Acetate (Anhydrous)
Acetic Acid(to adjust to pH 5)

4.2g
~400µl

Dissolve Sodium Acetate in 10ml of nanopure dH₂O. Add Acetic Acid to pH 5. Store refrigerated.

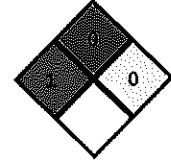
Date	Initials	Sodium Acetate Source/Lot#	Acetic Acid Source/Lot#	Lab Lot# Reagent Name
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT
				10xBRENT

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Form 116-BI

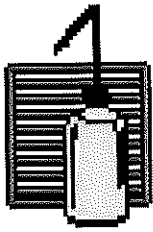
BRENTAMINE SOLUTION A



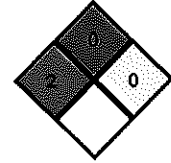
O-Dianisidine Tetrazotized (Fast Blue B Salt) 50 mg
10X buffer pH 5 5 ml

Dissolve Fast Blue B Salt in 5 ml of 10X Brentamine Buffer (Form 114-BI). Store refrigerated in a dark container.

Date	Initials	Fast Blue B Source/Lot#	10X Buffer Lab Lot#	Lab Lot# Reagent Name
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT
			10xBRENT	ABRENT



Form 118-BI



BRENTAMINE SOLUTION B

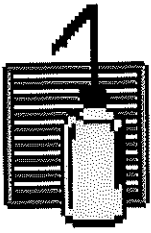
α -Naphthyl Phosphate (Disodium Salt)

50 mg

Dissolve in 5 ml of nanopure dH₂O. Store Refrigerated.

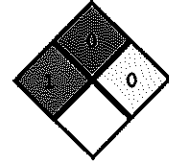
Date	Initials	α -naphthyl phosphate Source/Lot#	Lab Lot# Reagent Name
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT
			BBRENT

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Form 120-BI

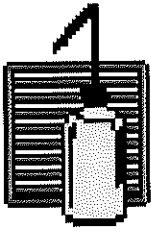
SALINE (0.85% NaCl)



NaCl 4.25g/500ml

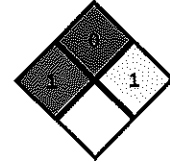
Dissolve the NaCl in 500 ml nanopure water. Sterilize by autoclaving.

Date	Initials	NaCl Source/Lot#	Lab Lot# Reagent Name
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl
			PNaCl



Form 122-BI

10X PHOSPHATE BUFFERED SALINE (PBS)

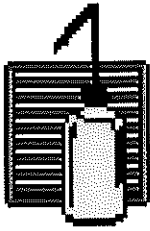


PBS 1 commercial pre-made packet

For 10X PBS, dissolve the package of powdered PBS in 1l of nanopure dH₂O. Check that pH \approx 7.4, autoclave, and store at RT.

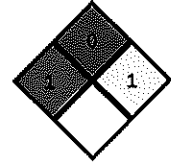
May also be purchased as 10X Solution.

Date	Initials	10X PBS Source/Lot#	Lab Lot# Reagent Name
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS
			10PBS



Form 124-BI

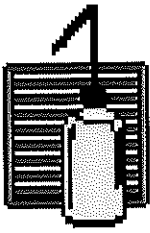
1X PHOSPHATE BUFFERED SALINE (PBS)



10X PBS Stock 100ml/l

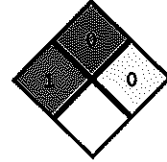
For 1l, mix 100ml 10X PBS (Form 122-BI) with 900ml of nanopure dH₂O, autoclave and store at RT.

Date	Initials	10X PBS Lab Lot#	Lab Lot# Reagent Name
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS
			PBS



Form 126-BI

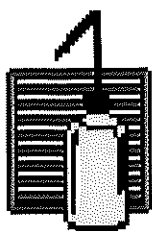
XMAS TREE STAIN SOLUTION A (Kernechtrot Solution)



Aluminum Sulfate 5g
Nuclear Fast Red 0.1g

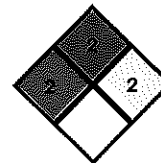
For 100ml, Dissolve the Aluminum Sulfate in 100ml **HOT** nanopure dH₂O. Immediately add the Nuclear Fast Red, mix, cool and filter (paper or ≥45µm). May be stored at RT.

Date	Initials	Aluminum Sulfate Source/Lot#	Nuclear Fast Red Source/Lot#	Lab Lot# Reagent Name
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA
				XMASA



Form 128-BI

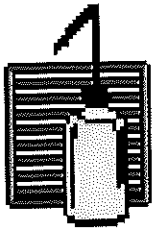
**XMAS TREE STAIN SOLUTION B
(Picroindigocarmine Solution)**



Saturated Picric Acid Solution 100ml
Indigo Carmine 0.33g

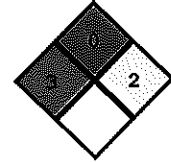
For 100ml, dissolve the Indigo Carmine in 100ml of the Picric Acid.
Mix and filter (paper or $\geq 45\mu\text{m}$). May be stored at RT.

Date	Initials	Picric Acid Source/Lot#	Indigo Carmine Source/Lot#	Lab Lot# Reagent Name
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB
				XMASB



Form 130-BI

NaOH 0.5N



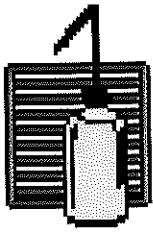
NaOH 5.0g/250ml

Dissolve the Sodium Hydroxide in 250ml sterile nanopure dH₂O.

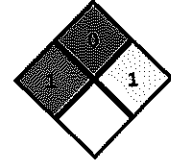
or

12.5ml of 10N (concentrated) NaOH added to 237.5ml sterile nanopure dH₂O.

Date	Initials	NaOH Pellets Source/Lot#	NaOH (Conc) Source/Lot#	Lab Lot# Reagent Name
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH
				1/2 NaOH



Form 132-BI



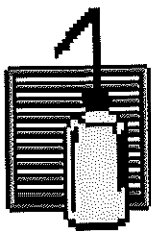
AMYLASE DIFFUSION BUFFER (pH6.9)

- NaH₂PO₄, anhydrous (0.045M) 2.7g
- Na₂HPO₄, anhydrous (0.055M) 3.9g
- NaCl (7mM) 0.2g

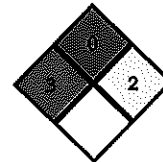
For 500ml, mix the above with 500ml dH₂O and store at RT.

Date	Initials	NaH ₂ PO ₄ Source/Lot#	Na ₂ HPO ₄ Source/Lot#	NaCl Source/Lot#	Lab Lot# Reagent Name
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB

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Form 134-BI

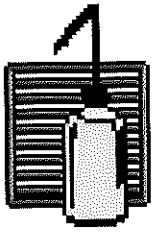


AMYLASE IODINE REAGENT

Potassium Iodide 1.65g
Iodine 2.54g

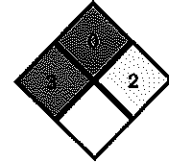
Add the above to 30ml nanopure dH₂O, mix well and store at 4°C.
Dilute 1:100 for Amylase Diffusion Test.

Date	Initials	KI Source/Lot#	I ₂ Source/Lot#	Lab Lot# Reagent Name
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR



Form 136-BI

SODIUM HYDROXIDE 5% (w/v)

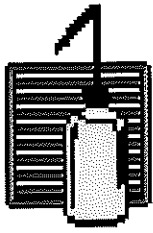


NaOH 5g/100ml nanopure dH₂O

Dissolve the NaOH in 100ml of nanopure dH₂O, mix well and store at RT.

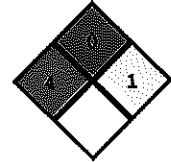
Date	Initials	NaOH Source/Lot#	Lab Lot# Reagent Name
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH
			5%NaOH

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Form 138-BI

MERCURIC CHLORIDE 10% (w/v)



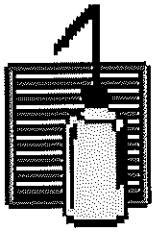
Mercuric Chloride

10g/100ml 95% EtOH

Dissolve the Mercuric Chloride in 100ml of 95% Ethanol, mix well and store at RT.

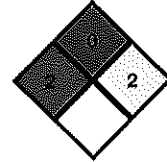
Date	Initials	EtOH Source/Lot#	Mercuric Chloride Source/Lot#	Lab Lot# Reagent Name
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC
				MC

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Form 140-BI

ZINC CHLORIDE 10% (w/v)



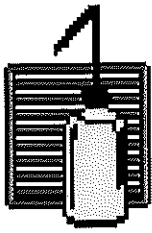
Zinc Chloride

10g/100ml 95% EtOH

Dissolve the Zinc Chloride in 100ml of 95% Ethanol, mix well and store at RT.

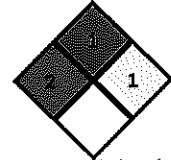
Date	Initials	EtOH Lot# Source/Lot#	Zinc Chloride Source/Lot#	Lab Lot# Reagent Name
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC
				ZC

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Form 201-BI

1M TRIS-HCl Buffer pH7.5



Tris Base (tris[Hydroxymethyl]amino methane)
or

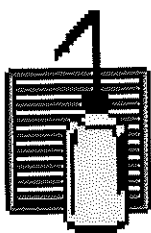
121.1 g

Tris-HCl (tris[Hydroxymethyl]amino methane hydrochloride)

157.6 g

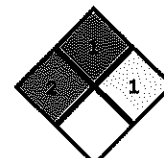
Dissolve Tris in ~800 ml nanopure dH₂O. Adjust to pH7.5. Q.S. to 1l with nanopure dH₂O, autoclave and store at RT.

Date	Initials	Tris Base Source/Lot#	Tris-HCl Source/Lot#	Lab Lot# Reagent Name
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5
				TRIS7.5



Form 203-BI

1M TRIS-HCl Buffer pH8



Tris Base (tris[Hydroxymethyl]amino methane)
or

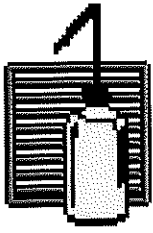
121.1 g

Tris-HCl (tris[Hydroxymethyl]amino methane hydrochloride)

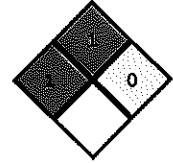
157.6 g

Dissolve Tris in ~800 ml nanopure dH₂O. Adjust to pH8. Q.S. to 1l with nanopure dH₂O, autoclave and store at RT.

Date	Initials	Tris Base Source/Lot#	Tris-HCl Source/Lot#	Lab Lot# Reagent Name
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8
				TRIS8



Form 205-BI



ETHYLENEDIAMINE TETRAACETIC ACID (EDTA) 0.5M

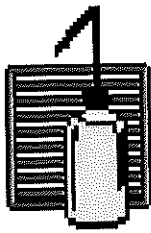
Na₂EDTA·2H₂O

186.1g/l

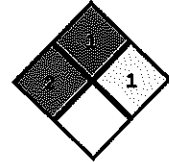
Dissolve EDTA in 800ml nanopure H₂O. Add ~20g of NaOH pellets to bring the pH to near 8.0. When fully dissolved adjust pH to 8.0 and bring final volume to 1l. Autoclave or filter sterilize; store at RT.

May also be purchased as 0.5M EDTA solution.

Date	Initials	EDTA Source/Lot#	NaOH Source/Lot#	Lab Lot# Reagent Name
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA
				EDTA



Form 207-BI

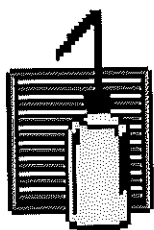


STAIN EXTRACTION BUFFER pH8
(10mM EDTA, 10mM Tris, 100mM NaCl, 2% SDS)

1M Tris-HCl, pH8	5ml
0.5M EDTA, pH8	10ml
5.0M NaCl	10ml
10% SDS	100ml

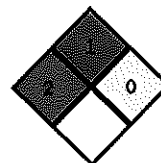
For 500ml, Mix the Tris, EDTA, NaCl and SDS with ~375ml nanopure dH₂O. Dispense into 50ml conical tubes and store at RT.

Date	Initials	Tris-HCl Source/Lot#	EDTA Source/Lot#	NaCl Source/Lot#	SDS Source/Lot#	Lab Lot# Reagent Name
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB
						SEB



Form 213-BI

TRIS/SODIUM CHLORIDE/EDTA (TNE)
(10mM Tris, 100mM NaCl, 1.0mM EDTA)



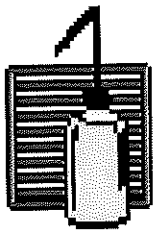
1M Tris-HCl, pH=8.0	5ml (0.61g Tris)
5M NaCl	10ml (2.92g)
0.5M EDTA, pH=8.0	1ml (0.19g)

For 500ml, mix the Tris-HCl, NaCl and EDTA with ~450ml nanopure dH₂O. Q.S. to final volume of 500ml, dispense into 50ml conical tubes, autoclave and store at RT.

Or

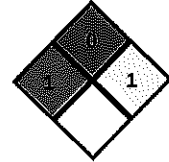
Mix solid Tris base, NaCl, and EDTA with ~400ml nanopure dH₂O. Adjust to pH8, bring to a final volume of 500ml, dispense into 50ml conical tubes, autoclave and store at RT.

Date	Initials	Tris Source/Lot#	NaCl Source/Lot#	EDTA Source/Lot#	Lab Lot# Reagent Name
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE
					TNE



Form 215-BI

N-LAUROYLSARCOSINE (SARKOSYL) 20%

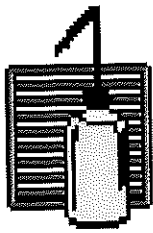


N-Lauroylsarcosine 10g/50ml

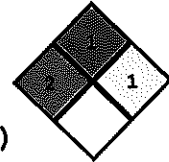
Dissolve the Sarkosyl in 50ml of nanopure dH₂O using gentle heat and mixing. Filter-sterilize and divide into 15ml conical tubes. Store at room temperature.

Date	Initials	Sarkosyl Source/Lot#	Lab Lot# Reagent Name
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S
			S

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Form 217-BI

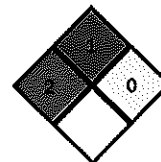
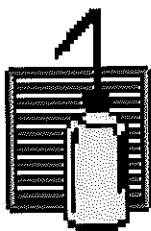


SPERM WASH BUFFER pH7.5
(10mM EDTA, 10mM Tris-HCl, 50mM NaCl, 2% SDS)

1M Tris-HCl, pH7.5	5ml
0.5M EDTA, pH8	10ml
5.0M NaCl	5ml
10% SDS	100ml

For 500ml, Mix the Tris-HCl, EDTA, NaCl and 10% SDS with ~380ml nanopure dH₂O. Dispense into 50ml conical tubes and store at RT.

Date	Initials	1M Tris-HCl Source/Lot#	0.5M EDTA Source/Lot#	5.0M NaCl Source/Lot#	10% SDS Source/Lot#	Lab Lot# Reagent Name
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB
						SWB



DTT (0.39M)

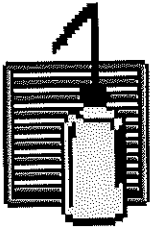
Dithiothreitol (DTT)

60.1mg/ml

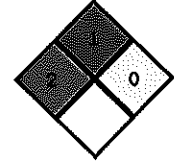
Dissolve the DTT in 1ml nanopure dH₂O. Aliquot ~100µl into microcentrifuge tubes. Store at ~-20°C.

Date	Initials	DTT Source/Lot #	Lab Lot# Reagent Name
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT
			DTT

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DTT (1M)



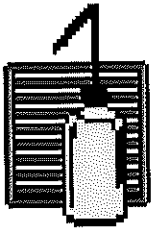
Dithiothreitol (DTT)

154.0mg/ml

Dissolve the DTT in 1ml nanopure dH₂O. Dispense ~100µl each into microcentrifuge tubes. Store at ~-20°C.

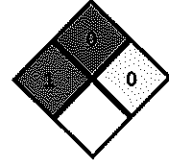
Date	Initials	DTT Source/Lot #	Lab Lot# Reagent Name
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT
			1MDTT

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Form 225-BI

CHELEX REAGENT 5%

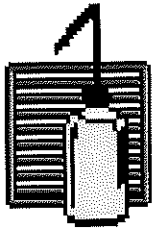


Chelex

0.5g/10ml

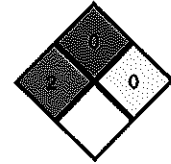
Dissolve the Chelex in 10ml sterile nanopure dH₂O. This solution should be freshly prepared prior to use and the remaining solution discarded after ≤3 days in refrigerator.

Date	Initials	Chelex Source/Lot#	Lab Lot# Reagent Name
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE
			CHE



Form 227-BI

LITHIUM CHLORIDE (LiCl) 8M



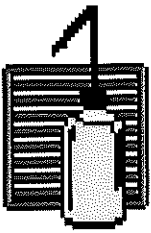
Lithium Chloride

16.96g/50ml

Dissolve the Lithium Chloride in 50ml nanopure dH₂O. Dispense into 15ml conical tubes. Sterilize by autoclaving and store at RT.

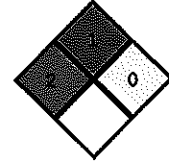
May also be purchased as 8M solution.

Date	Initials	LiCl Source/Lot#	Lab Lot# Reagent Name
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl
			LiCl



Form 229-BI

PCR-TE (TE⁻⁴) BUFFER
(10mM Tris-HCl, 0.1mM EDTA)

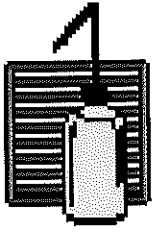


1M Tris-HCl, pH8
0.5M EDTA, pH8

5ml
0.1ml

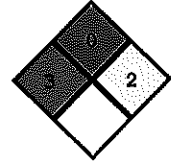
For 500ml, mix Tris-HCl and EDTA with 495ml nanopure dH₂O. Dispense into 50ml conical tubes, autoclave and store at RT.

Date	Initials	1M Tris-HCl Source/Lot#	0.5M EDTA Source/Lot#	Lab Lot# Reagent Name
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE
				TE



Form 231-BI

NaOH 5N

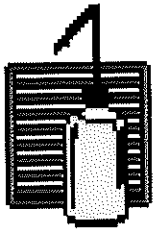


NaOH 50g/250ml

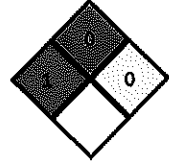
Dissolve the Sodium Hydroxide in 250ml sterile nanopure dH₂O.
or
125ml of 10N (concentrated) NaOH added to 125ml sterile nanopure dH₂O.

Date	Initials	NaOH Pellets Source/Lot#	NaOH (Conc) Source/Lot#	Lab Lot# Reagent Name
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH
				5NaOH

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Form 233-BI

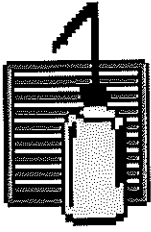


SODIUM CHLORIDE (NaCl) 5M

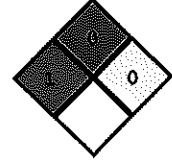
NaCl 146.1g/500ml

Dissolve the NaCl in 500 ml nanopure water. Sterilize by autoclaving.
May also be purchased as 5M solution. This may be diluted for 1M NaCl.

Date	Initials	NaCl Source/Lot#	Lab Lot# Reagent Name
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl
			5NaCl



Form 235-BI



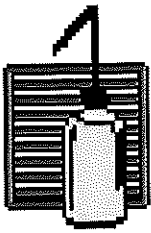
SALINE SODIUM CITRATE BUFFER (SSC) 2X

20XSSC (commercial purchase)

20ml

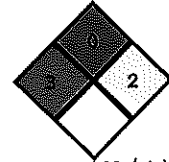
For 500ml, mix 50ml of SSC with 450ml of nanopure dH₂O and store at RT.

Date	Initials	20XSSC Source/Lot #	Lab Lot# Reagent Name
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC
			2XSSC



Form 237-BI

DENATURATION/SPOTTING SOLUTION
(0.5N NaOH, 0.5M NaCl)

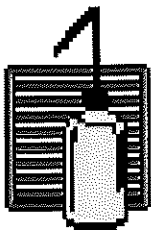


Sodium Hydroxide 20.0g/l (or 50ml of 10N NaOH/l)
Sodium Chloride 29.2g/l (or 100ml of 5M NaCl/l)

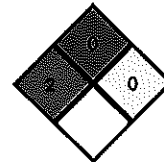
Add the NaOH and NaCl to ~800ml nanopure dH₂O. Q.S. to 1l with nanopure dH₂O, mix well and store at RT.

Date	Initials	NaOH Source/Lot#	NaCl Source/Lot#	Lab Lot# Reagent Name
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS
				DS

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Form 239-BI



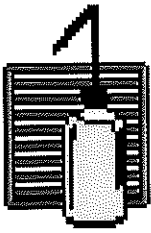
NEUTRALIZATION SOLUTION
(2X SSC, 0.2 M Tris-HCl, pH7.5)

20X SSC 100ml/l
1 M Tris-HCl (pH7.5) 200ml/l

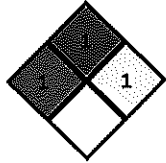
Add the SSC and Tris-HCl to 700ml nanopure dH₂O. Mix well and store at RT.

Date	Initials	20X SSC Source/Lot#	1M Tris-HCl Source/Lot#	Lab Lot# Reagent Name
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS
				NS

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Form 243-BI



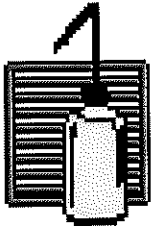
**HYBRIDIZATION SOLUTION
(5X SSPE; 0.5% SDS)**

10% SDS
20X SSPE

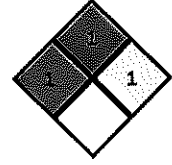
50ml
250ml

For 1l, mix the SDS and SSPE with 700ml nanopure H_2O .

Date	Initials	10% SDS Source/Lot#	20X SSPE Source/Lot#	Lab Lot# Reagent Name
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB
				QHB



Form 245-BI

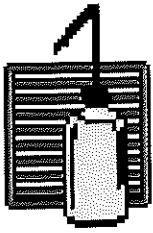


QUANTIBLOT WASH SOLUTION (QWS)
(1.5XSSPE, 0.5% SDS)

20X SSPE 150ml
10% SDS 100ml

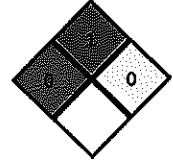
Mix the SSPE and SDS with 1750ml of nanopure dH₂O and store at RT. It may be necessary to warm before use to ensure solids remain in solution.

Date	Initials	20X SSPE Source/Lot#	10% SDS Source/Lot#	Lab Lot# Reagent Name
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS
				QWS



Form 247-BI

CITRATE BUFFER pH5, 0.1M (QCB)



200ml of 10X Citrate Buffer (commercial purchase)

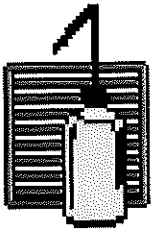
Mix 200ml of 10X Citrate Buffer (pH5) with 1800ml of nanopure dH₂O. Mix thoroughly. Check, and if necessary adjust, pH. Store at RT.

or

Trisodium Citrate Dihydrate (Na₃C₆H₅O₇•2H₂O) 36.8g
Citric Acid Monohydrate (C₆H₈O₇•H₂O) (~12g)

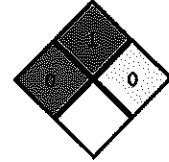
Dissolve 36.8g Trisodium Citrate Dihydrate in ~1600ml nanopure dH₂O. Adjust to pH5.0 (±0.2) by addition of Citric Acid Monohydrate (~12g). Adjust the final volume to 2l with nanopure dH₂O and mix thoroughly. Store at room temperature.

Date	Initials	10X Buffer Source/Lot#	Dihydrate Source/Lot#	Monohydrate Source/Lot#	Lab Lot# Reagent Name
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB
					QCB



Form 249-BI

BOVINE SERUM ALBUMIN (BSA) 4%



BSA 0.4 g
PCR-TE 10 ml

Dissolve the BSA in PCR-TE. Filter-sterilize and dispense ~500µl each into 1.5ml microfuge tubes. Store at ~-20°C.

Date	Initials	BSA Source/Lot#	PCR-TE Source/Lot#	Lab Lot# Reagent Name
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA
				BSA

DNA EXTRACTION WORKSHEET

Scientist _____

Case# _____

Blood/Saliva Extraction

Date _____

Items

- | | | |
|-----------------|--------------|-------|
| | <u>Lot #</u> | |
| 1a. 400µl SEB | SEB | _____ |
| 1b. 10µl Pro K | ProK | _____ |
| 2. 200µl Chelex | Che | _____ |
| 3a. 150µl FTA | FTA | _____ |
| 3b. 150µl TE | TE | _____ |

Hair Extraction

Date _____

- | | | |
|-----------------|-------|-------|
| 1a. 500µl SEB | SEB | _____ |
| 1b. 20µl 1MDTT | 1MDTT | _____ |
| 1c. 7.5µl Pro K | ProK | _____ |

Fresh Bone Extraction

Date _____

- | | | |
|----------------|------|-------|
| 1a. 400µl SEB | SEB | _____ |
| 1b. 10µl Pro K | ProK | _____ |
| 1c. 15µl LiCl | LiCl | _____ |

Old Bone Extraction

Date _____

- | | | |
|-----------------|------|-------|
| 1a. 3ml SEB | SEB | _____ |
| 1b. 100µl Pro K | ProK | _____ |
| 1c. 100µl LiCl | LiCl | _____ |

Ethanol Precipitation

Date _____

- | | | |
|--------------------|-------|-------|
| 1a. 500µl PCIAA | PCIAA | _____ |
| 1b. 1.0ml AbsEtOH | | |
| 1c. 1.0ml 70% EtOH | | |
| 1d. TE | TE | _____ |

Microconcentration

Date _____

- | | | |
|-----------------|-------|-------|
| 1a. 500µl PCIAA | PCIAA | _____ |
| 1b. TE | TE | _____ |

DIFFERENTIAL DNA EXTRACTION WORKSHEET

Scientist _____

Case# _____

Differential ♀ Extraction

Date _____

Lot #

Items

- 1a. 400µl TNE TNE _____
- 1b. 25µl Sarkosyl S _____
- 1c. 75µl H₂O _____
- 1d. 10µl Pro K ProK _____

Differential ♂ Extraction

Date _____

- 1a. 500µl SWB SWB _____
- 1b. 150µl TNE TNE _____
- 1c. 50µl Sarkosyl S _____
- 1d. 40µl 0.39MDTT DTT _____
- 1e. 150µl H₂O _____
- 1f. 10µl Pro K ProK _____

Ethanol Precipitation

Date _____

- 1a. 500µl PCIAA PCIAA _____
- 1b. 1.0ml AbsEtOH _____
- 1c. 1.0ml 70% EtOH _____
- 1d. TE TE _____

Microconcentration

Date _____

- 1a. 500µl PCIAA PCIAA _____
- 1b. TE TE _____

QUANTIBLOT WORKSHEET

Scientist _____

ImageID _____

Date: _____

Quantiblot Kit Lot# _____

Spotting Solution Lot# QSS _____

Membrane Source _____

Lot# _____

Hybridization Solution Lot# QHB _____

30% H_2O_2 Lot# _____

Wash Buffer Lot# QWS _____

Citrate Buffer Lot# QCB _____

Chemiluminescence Reagents Lot# _____

Record sample ID in table below. Include Image Station data and DNA Concentration Worksheet. Placement of standards may be varied by scientist.

Note: Image station defines whole column as 'standard' so no samples may be placed in a column with standards.

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

ACES WORKSHEET

Scientist _____

ImageID _____

Date _____

ACES™ Kit Lot# _____

Pre-wetting Solution Lot# 2XSS

Membrane Source _____

Lot# _____

Denaturation/Spotting Solution Lot# DS

Neutralization Solution Lot# NS

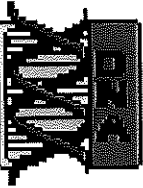
ACES™ Final Wash Buffer Lot# _____

Chemiluminescence Reagents Lot# _____

Record sample ID and ng detected in table below. Attach Image Station data. High and low standard need not be run (40ng, 0.04ng) and scientist may choose to change placement of standards.

Note: Image station defines whole column as 'standard' so no samples may be placed in a column with standards.

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 210-BI

STR AMPLIFICATION SET-UP

Date: _____ Scientist: _____ STR Kit Type: _____

STR Kit Lot: _____ Taq Lot: _____

Reagent	$\mu\text{l}/\text{sample}$	X	Master Mix #Samples	=	μl in Master
Rxn Buffer	_____ μl		_____		_____
Primers	_____ μl		_____		_____
H ₂ O	_____ μl		_____		_____
Taq Gold	_____ μl		_____		_____
Master Mix/Sample	_____ μl				_____ <u>Case (s) / Samples</u>
DNA Template	_____ μl				
Total Rxn Volume	_____ μl				
PCR TE Lot#	_____				

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Front



Form 212-BI

STR BLIND CONTROL GENOTYPE CHECK

Blind Control Number: _____ Scientist's Initials: _____

Date: _____

LOCUS	ALLELES	LOCUS	ALLELES
D3S1358		TH01	
D21S11		D18S51	
Penta E		D5S818	
D13S317		D7S820	
D16S539		CSE1PO	
Penta D		Amelogenin	
vWA		D8S1179	
TPOX		FGA	

Correct Genotype

Reviewer's Initials _____

Comments:



STR Technical Review Checklist

Case Number: _____ Reviewer's Initials: _____ Date: _____

Is the following paperwork included in the case file?

- Case Notes
- Extraction Worksheet
- Slot Blot Worksheet and Kodak Image Data
- DNA Concentration Worksheet
- Amplification Worksheet
- Genotyper Electropherogram Plots
- Allelic Table

Data Review:

- Correct assignment of size standard peaks (may be examined in Genescan or Genotyper).
- Positive Control appears as expected in Genescan.
- No allelic peaks or unacceptable artifacts found in Negative Controls.
- No unacceptable matrix problems (e.g., excessive pull-up or baseline problems).
- Correct genotypic assignment of ladder alleles.
- Sample plots examined for proper genotype and off-ladder assignments.
- Verify Genotypic result of positive and negative controls.
- Genotyper plot results and table results are in agreement.
- Conclusion(s) are supported by results.

Comments:



Form 306-BI

STR CODIS Review Checklist

CODIS Run: _____ Reviewer's Initials: _____ Date: _____

Is the following paperwork included in the CODIS Data file?

- Extraction Worksheet
- Amplification Worksheet
- Injection List (copy)
- Allelic Table

Data Review:

- Correct assignment of size standard peaks (may be examined in Genescan or Genotyper).
- Positive Control appears as expected in Genescan.
- No allelic peaks or unacceptable artifacts found in Negative Controls.
- No unacceptable matrix problems (e.g., excessive pull-up or baseline problems).
- Correct genotypic assignment of ladder alleles.
- Sample plots examined for proper genotype and off-ladder assignments.
- Verify Genotypic result of positive and negative controls.
- Genotyper plot results and table results are in agreement.
- Data certified for upload to CODIS.

Comments:

CODIS SAMPLE EXPUNGEMENT CHECKLIST

Date: _____ Initials _____ Date Completed: _____

Requesting Party: _____

Offender Number: _____

1. Make copy of Expungement Request/Order.
2. Offender Name found in DNA STiMAS Yes No
If no, contact requesting party both by phone and in writing. Document the contact and any resulting action(s).
3. Request Verified/Authenticated Yes No
If no, document the contact and any resulting action(s).
4. Criminal History Check
 Additional Qualifying Offense(s)? No Yes
If yes, contact requesting party both by phone and in writing. Document the contact and any resulting action(s).
5. DNA re-testing of original sample. Yes No
6. New DNA Sample submitted and tested? Yes No
If no, expungement will NOT be performed.
7. DNA Collection Report removed and marked "EXPUNGED".
8. Removal from DNA STiMAS.
9. Removal from CODIS.
10. Upload to NDIS.
11. Sample destroyed.

Scientist's Name (please print): _____ Initials _____

Witness's Name (please print): _____ Initials _____

12. All necessary documentation generated, sealed and distributed appropriately?



FORENSIC BIOLOGY EQUIPMENT MAINTENANCE/REPAIR RECORD

FILL IN ALL AVAILABLE INFORMATION.

Equipment Name/Description: _____

Serial Number: (if multiple, e.g., list all or reference)

Scheduled Maintenance/Repair (circle one)

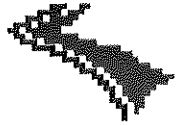
If repair, brief description of identified problem:

Vendor/Individual Performing Action: _____

Result: Completed / taken out of service / sent out for calibration/repair / other (circle one)

Comments:

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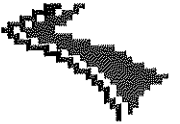
FORENSIC BIOLOGY WEEKLY QC

Form 404-QC

DATE	°C			°C			°C		
	Min	Max		Min	Max		Min	Max	
COMBO F/F A									
FRIDGE A									
FREEZER A									
FREEZER B									
FREEZER C									
COMBO F/F B									

DATE	°C			°C			°C		
NANOPURE/RO SYSTEM	Min	Max		Min	Max		Min	Max	
°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 HOODS																			
AIR FLOW	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
AUTOClave																			
WIPE DOOR GASKET																			
FLUSH STEAM GENERATOR																			
CHECK STERILIZATION																			
LABORATORY AND OTHER EQUIPMENT																			
EYE WASH STATION																			
PH METER																			
ARTTEL PCS2																			
CLEAN CENTRIFUGES																			
CLEAN PIPETS																			
PCR LAB CLEANED																			

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

1



FORENSIC BIOLOGY QUARTERLY QC

I. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 9700

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

Temperature Non-Uniformity

Table with 3 columns: SETPOINT VALUE, 94 °C, 37 °C. Rows include A1, A12, C4, C9, F4, F9, H1, H12, and summary rows for TNU at 94°C and 37°C.

Calibration Verification

Table with 4 columns: SETPOINT VALUE, 85°C, 45°C, PASS/FAIL. Row A6 shows FAIL.

II. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 480

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

Temperature Uniformity (File 32)

Table with 6 columns: SETPOINT VALUE, 95 °C, 40 °C, SETPOINT VALUE, 95 °C, 40 °C. Rows include A1, A3, A6, A8, C1, C3 with corresponding setpoint values like C6, C8, F1, F3, F6, 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)

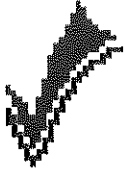
Prior to test #5 call up File #35 and run for 10 minutes.

Test #5: Sensor Test ____ (< 0.5°C)

Test #6: Overshoot ____ (<0.5°C)

Test #7: Undershoot ____ (<2.0°C)

PLACE DOCUMENT IN PCR QC BINDER



FORENSIC BIOLOGY QUARTERLY QC

Form 408B-QC

SCIENTIST: _____

QC DATE: _____

IV. Chemical Safety Shower Check _____

V. Forensic Biology Reagent Balance Check

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

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

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

VI. Centrifuge RPM Check

Centrifuge	Digital Readout	Tachometer Reading
A	_____	_____
B	_____	_____
C	_____	_____

Note: It is important for Centricon use that rpm/rcf readouts not be an underestimate of the true values. If tachometer readings exceed digital readouts or vary in either direction ($\leq 100rpm$) a notice should be placed on the instrument delineating the translation for value. If greater ($>100rpm$) differences are noted it may be necessary to have maintenance performed by a professional vendor.



QC ABACARD® HEMATRACE® KIT

HEMATRACE® KIT LOT: _____

DATE RECEIVED: _____

SCIENTIST: _____

QC DATE: _____

Perform test as usual with one 2mm² cutting and one 2mm thread from known bloodstain. Record results (include time it took for positive rxn to be visible). If available, attach photo documentation and place in Forensic Biology QC binder.

SAMPLE	RXN	TIME (min. sec.)
2mm ² cutting		
2mm thread		
Neg		

QA/QC PASSED: YES NO

Comments:

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QC OneStep ABACARD® p30 KIT

ABACARD® p30 KIT LOT: _____ DATE RECEIVED: _____

SCIENTIST: _____ QC DATE: _____

Perform test as usual with a known semen extract, as well as ~10ng/ml (10µl of a 1:500 dilution) and ~50ng/ml (10µl of a 1:100 dilution) of Seri Semen Standard. Record results (include time it took for positive rxn to be visible). If available, attach photo documentation and place in Forensic Biology QC binder.

SAMPLE	RXN	TIME (min. sec.)
Semen Extract		
10ng/ml		
50ng/ml		
Neg		
*250ng/ml or 1:10		

The semen extract must have a positive rxn within 10 minutes for passing. The Seri standards are used to estimate the range of sensitivity of the kit lot.

For the semen standard dilutions, if a positive rxn is not obtained at 10 minutes, continue to monitor and record result at the end of 15 minutes. In addition, *run a 250ng/ml (50µl of the 1:100 dilution to 150µl of extraction buffer) or a 1:10 dilution of the semen stain extract to ensure the kit is operating within reasonable limits for forensic identification. In addition to the neat semen extract, this control sample (250ng/ml or 1:10 extract) must result in a positive rxn within 10 minutes.

QA/QC PASSED: YES NO

Comments:



QC STR KITS

STR KIT: _____ DATE RECEIVED: _____

KIT MANUFACTURER: _____ KIT LOT #: _____

LAB LOT#: _____ SCIENTIST: _____ QA/QC DATE: _____

KIT COMPONENT	LOT NUMBER
PRIMER MIX	
REACTION MIX	
CONTROL DNA	
TAQ GOLD	
ALLELIC LADDER	

Perform extraction of one Blind Control and amplify as usual with reagent blank and controls.

Run Date: _____ Run Folder: _____

QA/QC PASSED: YES NO

Comments:

Attach the appropriate extraction/amplification/BC forms used and the Genotyper Electropherograms; place in Forensic Biology QC Binder. Note: A CODIS run may be used to validate STR kit.



ANNUAL NIST QC RUN

SCIENTIST: _____

QC DATE: _____

At a minimum of once a year, an 'in-date', certified NIST-SRM standard will be analyzed with our standard procedures. Blind Control samples may be analyzed simultaneously to 'certify' them.

The Genescan® and Genotyper® Data will be analyzed as usual and quality of results will be reflected in the comments or 'passed' areas. The Genotyper® Electropherograms and an Allele Table will be printed (for the NIST sample(s)) and stored in the Forensic Biology QC binder.

Run Folder: _____

QC PASSED: YES NO

Comments:

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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.

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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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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