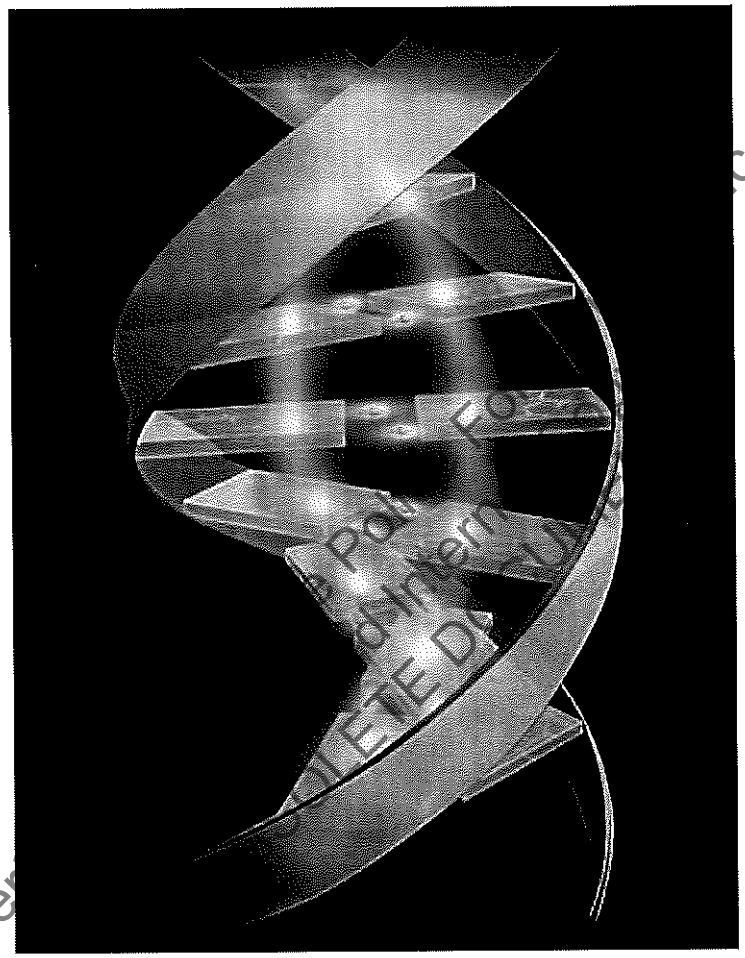
ISP FORENSIC BIOLOGY QUALITY/PROCEDURE MANUAL



October 2004

APPROVED BY:

Biology/DNA Supervisor Date: 11/9/04

Lab Improvement Manager Date: 11/23/04

BI-QA
Revision 3
10/2004

INTRODUCTION

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

1.0 STATEMENT OF PURPOSE AND OBJECTIVES

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

1.2 Objectives:

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

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

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

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

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

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

2.0 ORGANIZATION AND MANAGEMENT

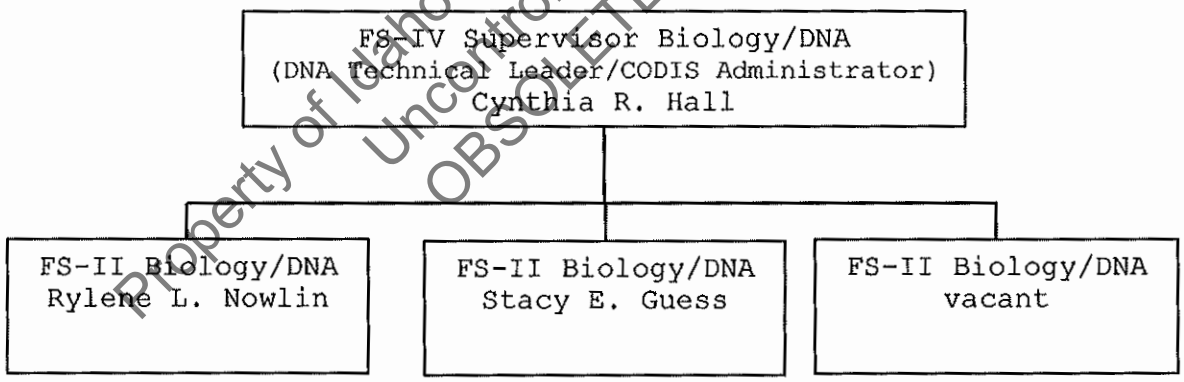
2.1 Organizational Chart and Functional Structure

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

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

2.2 Authority and Accountability in Forensic Biology

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



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

3.0 PERSONNEL QUALIFICATIONS AND TRAINING

3.1 Job Descriptions

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

3.2 Training

Refer to ISP Forensic Biology Training manual.

3.3 Qualifications

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

3.3.1 Forensic Biology/DNA Supervisor/Technical Manager

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

3.3.1.1 Education

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

3.3.1.2 Training

Training and experience in molecular biology and DNA-based analyses from academic, governmental, private forensic and/or research laboratory(ies).

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, quantification, and amplification set-up may be performed by appropriately trained laboratory technicians and/or those performing the biological screening of evidence following task-specific training and successful completion of a qualifying examination.

3.3.3.1 Education

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

3.3.3.2 Training

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

3.3.3.3 Experience

Must have a minimum of six months Forensic 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.

CYNTHIA R. HALL

TITLE: Forensic Scientist IV/Biology Supervisor

EDUCATION:

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

M.S., Molecular Genetics
San Jose State University

EXPERIENCE:

Job Title: Forensic Scientist IV/Biology Supervisor
Employer: Idaho State Police - Forensic Services
Tenure: 2/03 - present
Principal Duties: Direct supervision of biology unit personnel, including training and case management/assignment. Wrote and implemented a biology training manual for evidence screening and DNA analysis methods. DNA technical lead and CODIS state administrator. DNA analysis and screening of casework evidence and participate in the crime scene response program. Conduct periodic review, maintenance, and revisions of current biology SOP's. Research and validate new methodologies. Perform administrative and technical review of biology case reports. Provide and participate in training of law enforcement and medical personnel. Serve as an ASCLD/LAB inspector.

Job Title: Forensic Scientist III
Employer: Idaho State Police - Forensic Services
Tenure: 6/01 – 2/03
Principal Duties: Perform DNA analysis and screening of casework evidence and participate in crime scene response. Assist in the development and implementation of analysis and quality assurance SOP's for the biology unit. Serve as the lead for the unit and act as trainer/mentor for biology personnel. Perform administrative and technical review of biology casework. Provide training for law enforcement and medical personnel.

Job Title: Supervising Criminalist
Employer: Santa Clara County District Attorney's Crime Laboratory
Tenure: 7/99 – 6/01
Principal Duties: Direct supervision of biology/DNA employees, including training and case management/assignment. Creation/Implementation of a biology training manual for evidence screening and DNA analysis methods. DNA analysis and screening of casework evidence and participate in the crime scene response program. Conduct periodic review, maintenance, and revisions of current biology SOP's. Perform and oversee the validation, troubleshooting, and implementation of additional STR loci for use in casework. Serve as training coordinator/supervisor for the biology/DNA unit, instructor of law enforcement and medical personnel, DAB and ASCLD/LAB inspector, local CODIS administrator, and laboratory safety officer.

Job Title: Criminalist III
Employer: Santa Clara County District Attorney's Crime Laboratory
Tenure: 12/98 – 7/99
Principal Duties: Biology/DNA casework, latent fingerprint processing of related evidence, and participation in the crime scene response program. Perform peer/technical review of biology/DNA case reports. Lead analyst for the validation, troubleshooting, implementation, and training of STR's for use in casework. Serve as a mentor for new analysts and trainer of law enforcement and medical personnel. Act as the laboratory safety officer and local CODIS administrator.

Job Title: Criminalist II
Employer: Santa Clara County District Attorney's Crime Laboratory
Tenure: 6/95 – 12/98
Principal Duties: Serology (biological screening) and DNA casework responsibilities. Assist in validation and implementation of DNA methods for casework. Participate in crime scene response, training law enforcement personnel, and latent fingerprint processing of evidence. Serve as laboratory safety officer and local CODIS administrator.

Job Title: Laboratory Technician/DNA Analyst
Employer: California Department of Justice, DNA Laboratory
Tenure: 6/94 – 6/95
Principal Duties: Receipt and preservation of convicted offender blood samples submitted for entry into CODIS. DNA typing of offender/databank bloodstains using RFLP and PCR. Assist in the validation of DNA methods.

Job Title: Criminalist Intern
Employer: San Diego Police Department Crime Laboratory
Tenure: 3/93 – 3/94
Principal Duties: Screening of sexual assault evidence for the location and identification of semen.

ADDITIONAL TRAINING:

1993 In-service training: Serology (SDPD)

6/94 In-service training: DNA/RFLP (CA DOJ)

7/94 Forensic Serology – CA Criminalistics Institute

8/94 Polymerase Chain Reaction – CA Criminalistics Institute

8/94 In-service training: DNA/PCR-DQα (CA DOJ)

9/94 Forensic Sciences Overview – CA Criminalistics Institute

5/95 Workshop: DNA/D1S80 (CA DOJ)

5/95 Professional Meeting: DNA (CAC)

6/95 Crime Scene Investigation I – CA Criminalistics Institute

9/95 Electrophoresis and Isoelectric Focusing – CA Criminalistics Institute

10/95 Professional Meeting: DNA (Promega 6th International Symposium)

S 1996 Introduction to Statistics – UC Berkeley

3/96 Courtroom Presentation of Evidence – CA Criminalistics Institute

5/96 Professional Meeting (program co-chair): DNA & general Criminalistics (CAC)

5/96 Workshop: Bullet Path Reconstruction (CAC/SCCCL)

7/96 Workshop: Night Photography (SCCSO/SCCCL)

9/96 Workshop: Statistics (Promega 7th International Symposium)

1997 Training/Collaboration: STR Analysis and Instrumentation (ABD-HID)

5/97 Forensic Statistics on DNA Analysis – CA Criminalistics Institute

6/97 Short Tandem Repeat Analysis – CA Criminalistics Institute

2/98 Professional Meeting: DNA (AAFS)

7/98 Forensic Statistics – CA Criminalistics Institute

9/98 In-service Training – Latent Fingerprint Processing (SCCCL)

1/99 CODIS Local training – FBI/SAIC

8/99 Professional Meeting: DNA (ISFH 18th International Congress)

10/99 Crime Scene Reconstruction – Tom Bevel and Ross Garner

12/99 In-service training: DNA/STRs - ABI 377/310 (Applied Biosystems at SCCCL)

1/00 ASCLD/LAB Inspector training – ASCLD/LAB

2/00 Professional Meeting: DNA & general Criminalistics (AAFS)

4/00 Professional Meeting: Management (CACLD)

11/00 Professional Meeting (program chair): Management (CACLD)

2/01 Professional Meeting: DNA/CODIS User's Forum (FBI)

2/01 Workshop: DNA/DAB Audit training (FBI)

2/01 Professional Meeting: DNA & general Criminalistics (AAFS)

5/01 Professional Meeting: DNA (CAC)

94-01 Study Group Meetings: Biology/DNA (CAC)

10/01 Professional Meeting: DNA (Promega 12th International Symposium)

2/03 Professional Meeting: DNA & general Criminalistics (AAFS)

5/03 Professional Meeting: DNA & CODIS (CODIS State Admin.)

6/03 Professional Meeting: DNA (NIJ)

9/03 Bloodstain Pattern Analysis – Jeff Gurvis (FBI)

9/03 Professional Meeting: DNA (Promega 14th International Symposium)

9/03 Workshop: Y-STRs (Promega 14th International Symposium)

9/03 Workshop: Popstats (Promega 14th International Symposium)

10/03 In-Service Training: Krimesite Imager (Sirchie at ISP)

11/03 Professional meeting: DNA & CODIS (CODIS/CODIS State Admin.)

2/04 Crime Scene Survey, Documentation, & Diagramming – FBI

4/04 Workshop: Hair Examination for the DNA Analyst (NWAFS)

5/04 Professional Meeting: DNA & CODIS (CODIS State Admin.)

6/04 In-Service Training: ABI Prism 7000 (ABI at ISP)

6/04 Professional Meeting: DNA (NIJ)

Professional Organizations:

American Academy of Forensic Sciences (AAFS) – Member (2/95 - present)

California Association of Criminalists (CAC)– Member (10/95 – 12/03)

American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB) – Inspector (1/00 – present)

California Association of Crime Lab Directors (CACLD)- Member (11/99 - 12/01)

Association for Crime Scene Reconstruction (ACSR)- Member (2/00 – 12/02)

Teaching Experience:

Crime Scene Processing and DNA Evidence – Trainer for law enforcement and medical personnel (1994-2001)

Homicide Investigation/DNA Evidence – Instructor, San Jose State University, Administration of Justice (11/95, 5/96)

Evidence Technician Course/DNA Evidence – Instructor, Evergreen College (10/98, 5/01)

Crime Scene Processing and DNA Evidence, 8hr portion of Robert Presley Institute of Criminal Investigation Core Course – Instructor, San Jose State University, Administration of Justice (1997-1999)

Crime Scene Processing/Biological Evidence Collection and Packaging, 2 hour classroom and 3 hour crime scene practical – Instructor, Idaho State Police Basic POST Academy (2002-present)

Research, Publications, Presentations:

Validation and Casework Experiences at Santa Clara County Using Profiler Plus – Presentation 6/24/98 (CAC Study Group Meeting)

STR Data Interpretation – Presentation 10/1/98 (CAC Study Group Meeting)

STR Validation, Issues Involving the Use of Chelex and Kit Volume Reduction – Presentation (CAC Spring Seminar 10/98)

Hall, C.R., et al. 2000. "Validation of the AmplFlSTR Profiler Plus and the AmpFlSTR COfiler PCR Amplification Kits, Using TWGDAM Guidelines." – AAFS Annual Meeting Proceedings, Abstract B77.p.56 (AAFS Meeting 2/00)

Hall, C.R. 2000. "Effects On Stutter Production During Amplification of Tetranucleotide STR Loci." – Master's Thesis (San Jose State University)

Effects Of Locus, Sequence, and Primers on Stutter Production During PCR Amplification of Tetranucleotide Short Tandem Repeat Loci. – Presentation (CAC Spring Seminar 5/01)

Rylene L. Nowlin

TITLE: Forensic Scientist II

EDUCATION:

B.S., Biology and History
Albertson College of Idaho

M.S., Biology
Boise State University
(To be completed Fall 2004)

EXPERIENCE:

Job Title:	Forensic Scientist II
Employer:	Idaho State Police - Forensic Services
Tenure:	11/02 - present
Principal Duties:	Perform biological screening of casework evidence and administrative and technical review of biology screening casework. Participate in crime scene response and training of law enforcement and medical personnel.

ADDITIONAL TRAINING:

2/03	In-service training: Biology Screening (ISP)
3/03	Bloodstain Pattern Analysis – Jeff Gurvis (FBI)
4/03	Forensic Digital Imaging – David "Ski" Witzke
5/03	Courtroom Presentation of Evidence – Raymond Davis
8/03	Crime Scene Survey, Documentation and Diagramming – Richard Berry (FBI)
9/03	Professional Meeting: DNA (Promega 14th International Symposium)
9/03	Workshop: Y-STRs (Promega 14th International Symposium)
9/03	Workshop: Statistics (Promega 14th International Symposium)
8/04	PopStats – Arthur Eisenberg (UNT) and John Planz (UNT)
10/04	Workshop: Molecular Biology (Promega 15th International Symposium)
11/04	Genetic Typing Methods in Forensic Science – Terry Spears (CCI)

Teaching Experience:

Crime Scene Processing/Biological Evidence Collection and Packaging, 2 hour classroom and 3 hour crime scene practical – Instructor, Idaho State Police Basic POST Academy (2003-present)

STACY E. GUESS

TITLE: Forensic Scientist I/Biology

EDUCATION: B.S., Microbiology
University of Idaho

M.S.F.S., Forensic Science
University of Alabama at Birmingham

EXPERIENCE:

Job Title: Forensic Scientist I/Biology
Employer: Idaho State Police - Forensic Services
Tenure: 8/03 - present
Principal Duties: Perform biological screening of casework evidence. Participate in crime scene response. Prepare reagents and conduct quality control checks in the laboratory. Enter convicted offender buccal samples into computer based tracking system for CODIS.

Job Title: DNA Database Technician
Employer: Alabama Department of Forensic Sciences - CODIS
Tenure: 9/01 - 4/03
Principal Duties: Enter convicted offender samples into computer based tracking system for CODIS. Preserve convicted offender blood samples. Perform DNA analysis of convicted offender samples.

ADDITIONAL TRAINING:

1/02 In-service training: DNA Database/CODIS (ADFS)

2/02 Professional Meeting: Criminalistics & DNA (AAFS)

3/02 In-service training: DNA/STRs (ADFS)

7/02 In-service training: Capillary Electrophoresis & the ABI Prism 3100 (Applied Biosystems at ADFS)

2/03 Professional Meeting: Criminalistics & DNA (AAFS)

9/03 Evidence Handling (ISP)

12/03 Bloodstain Pattern Analysis Workshop (Utah Bureau of Forensic Services)

2/04 Crime Scene Survey Documentation & Diagramming (FBI)

4/04 Workshop: Hair Examination for DNA Analysts (NWAFS)

8/04 In-service training: Biology Screening (ISP)

Professional Organizations:

American Academy of Forensic Sciences (AAFS) – Student Member (2/02 - present)

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

Name of Lab: Forensic Investigation Center
Building 30
1220 Washington Avenue
Albany, NY 12226

Date: February 1, 2003

Name: Allison Yvonne Eastman **Job Title:** Supervisor DNA Services

Discipline(s): Indicate all areas in which you do case work.

___Controlled Substances
___Firearms/Toolmarks
___Trace Evidence
___Latent Prints
___Other:

___Toxicology
_X_Serology
_X_DNA
___Questioned Documents

Please list all sub-disciplines in which you perform casework:

Education: List all higher academic institutions attended:

Institution	Dates Attended	Major	Degree Completed
• Albany Medical College	1978 – 1984	Microbiology/ Immunology	Ph.D.
• SUNY Albany	1973 – 1975	Biology/Chemistry	B.S.
• Junior College Of Albany	1972 – 1973	Liberal Arts	A.A.S.

Other Training: List continuing education, workshops, in-service and other formal training received.

- December 11, 2002 — Applications of the Forensic Light Source. Full day seminar and hands-on workshop provided by Mr. George Setola, SPEX Instruments. NYSP Academy and NYSP Forensic Investigation Center
- July 25-26, 2002 — STR DNA Analysis and Troubleshooting, workshop hosted by Applied Biosystems, New York State Police Academy,. Albany NY.
- April 11-12, 2002 — *Use of the DNA Audit Document*, Federal Bureau of Investigation and NYS Division of Criminal Justice Services, Office of Forensic and Victim Services, Saratoga, NY.
- July 23-August 3, 2001 — *Mitochondrial DNA Analysis Workshop*, Federal Bureau Of Investigations, Quantico, VA.
- June 12 -14, 2001 — *DNA Advisory Board Audit Document Workshop*, Federal Bureau Of Investigations, Quantico, VA.
- May 30 – 31, 2001 — *Laboratory Accreditation Awareness Training* ISO/IEC 17025 *Workshop,* Quenpro International, NYSP Forensic Investigation Center.
- August 10 – 11, 2000 — *Effective Use Of DNA In Capital Cases*, New York Prosecutors Training Institute Summer College For District Attorneys, Syracuse, New York.
- October 13-17, 1999 — *Quality Assurance Workshop*, Northeastern Association Of Forensic Scientists 25th Annual Meeting, Hyannis, MA.

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

• April 27, 1999	*Capillary Electrophoresis Of Short Tandem Repeats*, NYS Department Of Justice And New York Commission On Laboratory Accreditation And Certification sponsored workshop given by PE/ABI, Westchester County Forensic Laboratory, Valhalla, New York.
• 1999	*Statistics Training*: A series of six (6) one (1) hour lectures given by James Jaccard, Ph.D., SUNYA.
• October 14, 1998	*Applications Of PE Biosystems Technology for Forensic Analysis*, Perkin Elmer Applied Biosystems STR Training Workshop, NYSP Forensic Investigation Center.
• August 17-21, 1998	*Forensic Hair and Fiber Microscopy*: a course of instruction by the McCrone Research Institute, NYSP Forensic Investigation Center.
• 1998:	*The Science of Forensic STR Analysis and Data Interpretation,* American Academy of Forensics.
• Nov. 23-25, 1997	*American Society of Crime Lab Directors Laboratory Accreditation Board Inspector Training Workshop*, NYSP Forensic Investigation Center.
• Oct. 21-23, 1997	*Statistics Training of DNA Scientists Workshop.*
• Oct. 15-16, 1997	*The Art of Giving Expert Testimony Workshop*, Northeastern Association Of Forensic Scientists 23rd Annual Meeting.
• 1996	*Principals of Statistical Interference I*, SUNYA Graduate Studies, Albany, New York.
• 1996	*Preparing And Submitting Expert Testimony Workshop*, Albany Law School, Albany New York.
• 1996	*Forensic DNA Testimony Workshop*, Mid-Atlantic Association Of Forensic Scientists, Harrisburg, PA.
• July 29 – Aug.2, 1996	*Human Remains Recovery Workshop*, NYSP And U.S. Army Central Identification Laboratory, Hawaii.
• 1993	*Forensic DNA Amplification Workshop*, PCR Information Network, Roche Molecular Systems, Inc., Alameda, CA.
• 1984 – 1986	Postdoctoral fellowship, *Immunogenetics*, NYS Department Of Health, Wadsworth Center, Albany, New York. Supervisor: Dr. Lorraine A. Flaherty.

Courtroom Experience: List the discipline(s) in which you have qualified to testify as an expert witness and indicate over what period of time and approximately how many times you have testified in each.

- Biological Science (Serology and DNA)
- *People of the State of New York v.:*
    - Chester Stanton (Sullivan county, 2002)
    - Nache Africa (Monroe County, 2002)
    - Jeffrey Clark (Grand Jury, Madison County 2001)
    - Jeffrey Canale (Warren County County, 2001)
    - Nache Africa (Grand Jury Monroe County, 2001)
    - Joseph Stuemer (Orange County, 2000)
    - John Doe (Grand Jury Monroe County, 2000)
    - Harold Mabee (Orange County County, 2000)
    - Anders Junger (Saratoga County, 2000)
    - Fernando Castellano (Albany County, 2000)
    - Isa W. Gray (Essex County, 2000)
    - John Patterson (Dutchess County, 2000); (St. Lawrence County, 2000)
    - Charles Saunders (Albany County, 2000)

- Hasheen Herring (Broome County, 2000)
- James Folsom (Orange County, 2000)
- Anthony Palmer (Dutchess County, 1999)
- Christopher Bristol (Oneida County, 1999)
- Lamont Hughes (Rensselaer County, 1999)
- Christopher Bush (Wayne County, 1998)
- Roosevelt Kindred (Albany County, 1998)
- Alan Johnson (Albany County, 1999)
- William McGovern, Jr. (Grand Jury Saratoga County, 1999)
- Dwayne Douglas (Rensselaer County, 1999)
- Fred Giguere (Clinton County, 1999)
- Scott McKenna & William Bonanni (Albany County, 1999)
- Colleen Shannon (Oneida County, 1999)
- Shonnel Burns (Rensselaer County, 1999)
- David Brown (Warren County, 1999)
- Douglas Getman (Jefferson County, 1999)
- Tracy Cross (Essex County, 1999)
- Alan Johnson (Albany County, 1998)
- Brian Martinez (Cayuga County, 1998)
- Christopher Bush (Wayne County, 1998)
- Christopher Hempstead (Grand Jury, Albany County, 1998)
- Nicholas Pryor (Albany County, 1998)
- Robert McCullough (Monroe County, 1998)
- Corey Young (Albany County, 1998)
- Vincent Zeh (Ulster County, 1998)
- Stanley Washington (Monroe County, 1998)
- Christopher Stecker (Broome County, 1998)
- Gerald O. Austin (Grand Jury, Madison County, 1998)
- Paul Roach (St. Lawrence County County, 1998)
- Sherman Brody And Alonzier McFadden (Rockland County, 1998)
- Daryl McNeil (Broome County, 1997)
- Gregory Cole (Genesee County, 1997)
- Christopher Stecker (Grand Jury, Broome County, 1997)
- Henry West (Schoharie County, 1997)

Professional Affiliations: List any professional organization of which you are or have been a member. Indicate any offices or positions held and the date(s) of these activities.

• 2003-present	Scientific Working Group for DNA Analysis Methods, Subcommittee on Expert DNA Review Systems
• 2002 – Present	Scientific Working Group for DNA Analysis Methods, Subcommittee on Mitochondrial DNA.
• 2001 – Present	Certificate Of Qualification to be a Laboratory Director In Forensic Biology, New York Department Of Health Clinical Laboratory Evaluation Program.

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

- 2001 – Present — Technical Working Group For Forensic Education and Training (TWGED), member of the Planning Panel and Co-Chair of the Committee For Undergraduate Curricula. National Institute of Justice and the University of West Virginia.
- 2001 – Present — Scientific Working Group for DNA Analysis Methods, Subcommittee on DNA Laboratory Safety, Federal Bureau Of Investigation, Quantico, VA, July 11-13.
- 1998 - 2000 — American Academy of Forensic Scientists (AAFS), provisional member in Criminalistics.
- 1998 – Present: — American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD/LAB®) Inspector.
- 1996 -Present — Northeastern Association Of Forensic Scientists (NEAFS).
- 1994 – 1998 — Inspector in Molecular Pathology, College of American Pathologists (CAP) Laboratory Accreditation Program..
- 1994 – 1997 — American Heart Association (AHA) Council Member.
- 1990 – 1996 — NYS Department of Health Certification of Qualification in Cellular Immunology.
- 1990 – 1996 — Association Of Medical Laboratory Immunologists (AMLI).

Employment History: List all scientific or technical positions held, particularly those related to forensic science. List current position first. Give a brief summary of principal duties in each position.

Job Title: Associate Professor of Biology **Employer:** State University of New York at Albany

Principal Duties: Development of MS in Forensic Biology program, teaching of Forensic courses and Forensic Biology Laboratory.

Tenure: 2002 - Present

Job Title: Supervisor of DNA Services **Employer:** New York State Police Forensic Investigation Center

Principal Duties: Training of new Forensic Scientists in forensic serology and DNA analysis; collaborative development of NYSP-SUNYA Masters of Science Degree track in Forensic DNA Profiling; supervision of forensic casework validation studies; analysis of select criminal cases.

Tenure: 2000 - Present

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

Job Title: Forensic Scientist III

Employer: New York State Police
Forensic Investigation Center

Principal Duties: Developed a PCR-DNA profiling service; QA/QC in PCR; PCR Training of analysts; casework; lectures in DNA evidence collection and preservation to the law enforcement community.

Tenure: 1995 - 2000

Job Title: Adjunct Assistant Professor

Employer: Albany Medical College
45 New Scotland Avenue
Albany, NY 12208

Principal Duties: Teaching of graduate and medical students and medical residents.

Tenure: 1995 - 1999

Job Title: Serologist II

Employer: Albany Medical College
45 New Scotland Avenue
Albany, NY 12208

Principal Duties: Part Time Job; off hours testing of potential organ transplant donors for possible infectious diseases.

Tenure: 1991 - 1997

Job Title: Laboratory Director

Employer: Department of Pathology and Laboratory Medicine Division of Experimental Pathology, A-81,
Albany Medical College,
47 New Scotland Avenue,
Albany, NY 12208

Principal Duties: Developed and directed a clinical laboratory service in molecular pathology; directed pathology graduate students; teaching of graduate and medical students and residents in molecular diagnostics and molecular pathogenesis; basic research on the molecular pathogenesis of cardiovascular disease.

Tenure: 1989 - 1995

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

Job Title: Research Scientist I

Employer: New York State Department of Health
Wadsworth Center for Laboratories and Research
P.O. Box 509
Albany, NY 12201-0509

Principal Duties: Development of recombinant DNA vaccines for clinical and agricultural applications.

Tenure: 1986 - 1989

Job Title: Postdoctoral Fellow

Employer: New York State Department of Health
Wadsworth Center for Laboratories and Research
P.O. Box 509
Albany, NY 12201-0509

Principal Duties: Research on the immunology and molecular biology of the transplantation genes and what role they play in controlling cancer induction and progression.

Tenure: 1983 - 1986

Job Title: Senior Technician

Employer: Department of Microbiology and Immunology
Albany Medical College
47 New Scotland Avenue
Albany, NY 12208

Principal Duties: Supervisor of a research laboratory involved in the study of cellular immunity; the characteristics, regulation, and pathobiology of the immune response.

Tenure: 1975 - 1978

Other Qualifications: List below any scientific publication and/or presentation you have authored or co-authored, research in which you have been involved, academic, or other teaching positions you have held, and any other information which you believe relevant to your qualification as a forensic scientist.

- Teaching/Presentations/Professional Activities
  - 1996: Human Identification by DNA Analysis – Location and Recovery of Human Remains. NYS Police and United States Army Central Identification Laboratory
  - 1997: *Collection and Preservation of DNA Evidence:* NYSP Bureau of Criminal Investigation (BCI) Training School.

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

- 1997: *DNA as an Investigative Tool* – Location and Recovery of Human Remains. NYS Police and United States Army Central Identification Laboratory, Hawaii.

- 1998: Technical Working Group for DNA Analysis Methods (TWGDAM), FNI Training Academy, Quantico, VA. Representative from the New York State Police, January 10 – 14. Member of the Subcommittee on DNA Evidence Collection manual.

- 1998: ASCLD/LAB® inspection of the Los Angeles Police Department Crime Laboratory. Inspector of Serology/DNA Section. March 28 – April 4.

- 1998: DNA-Capital Trial Advocacy Training Program; New York Prosecutors Training Institute, Inc. Reviewer of DNA Expert Testimony, October 26-27.

- 1998: *Fingerprints in Blood: DNA Technology in Forensic Identification,* Sigma Xi, The Scientific Research Society, Vassar College, Poughkeepsie, NY.

- 1999: ASCLD/LAB® inspection of the Georgia Bureau of Investigation Laboratory System. Inspector of Serology/DNA, Atlanta; Site Leader, GBI Augusta Crime Laboratory, January 10-15.

- 1999: *Biological Evidence:* Police crime scene and evidence specialist school, New York State Division of Criminal Justice Services, April 2.

- 1999: *Consultant on Forensic DNA Analysis,* State of New York Office of Attorney General Eliot Spitzer: Victor Ortiz V. State of New York, Claim #96390. Assisted Mr. Dewey Lee, Assistant Attorney General.

- 2000: ASCLD/LAB® inspection of the California Department of Justice Bureau of Forensic Services Laboratory System. Inspector of Serology/DNA at the Sacramento, Santa Rosa, Ripon, and French Camp Laboratories, January 8-16, 2000.

- 2000: *DNA as an Investigative Tool;* Crime Scene Specialist Workshop, New York State Department of Criminal Justice Services and the Capital District Forensic Officers Group, May 26.

- 2000: *Understanding the Scientist's Complete Case File*, New York Prosecutors Training Institute Summer College for District Attorneys. Effective use of DNA in Capital Cases, Syracuse, NY; August 10-22.

- 2000: *Consultant on Forensic DNA Analysis*, State of New York Office of Attorney General Eliot Spitzer: Vincent Jenkins v. State of NY, Claim #OAG-#00-001812-O. Assisted Ms. Leslie A. Stroth, Assistant Attorney General.

- 2000: *DNA Profiling Update*, New York State Association of County Coroners and Medical Examiners, Fall 2000 Conference: Back to the Basics, September 15-17, 2000.

- 2001: *DNA Databank Validation of a 16 locus STR System:* STR Validation Workshop, 11th International Symposium on Human Identification, sponsored by Promega Corporation; Biloxi, Mississippi; October 10.

- 2001: *Forensic Biology.* 26th Annual Meeting of the Northeastern Association of Forensic Scientists. Moderator. October 12.

- 2001: *DNA as an Investigative Tool;* Crime Scene Specialist Workshop, New York State Department of Criminal Justice Services and the Capital District Forensic Officers Group, December 5.

- 2001: ASCLD/LAB® inspection at the San Bernardino Sheriff's Scientific Investigation division. Inspector of Forensic Biology section. January 14-19.

- 2001: *DNA as an Investigative Tool;* Crime Scene Specialist Workshop, New York State Department of Criminal Justice Services and the Capital District Forensic Officers Group, March 20.

- 2001: *DNA as an Investigative Tool;* Crime Scene Specialist Workshop, New York State Department of Criminal Justice Services and the Capital District Forensic Officers Group, April 10.

- 2001: *DNA as an Investigative Tool;* Bureau of Criminal Investigation Training Workshop, New York State Police Academy, June 8.

- 2001: Admissions Committee, Department of Biology, Graduate Track in Forensic Molecular Biology, State University of New York at Albany, Albany, NY.

- 2001: Invited Co-auditor: New York State Department of Health Forensic Laboratory Evaluation Program Audit of Reliagene, Inc., New Orleans LA, October 18-19.

- 2001: *Forensic Biology,* seminar presented at the University at Albany, Department of Biology, November 2.

- 2002: *Update on the WTC Initiative at the New York State Police Forensic Investigation Center,* Scientific Working Group for DNA Analysis Methods (SWGDAM), Federal Bureau of Investigation, Quantico, VA; January 14-17.

- 2002: Bio 575: *Forensic Biology Laboratory* (2 credits), University at Albany, NYSP East Campus Training and Research Facility, Rensselaer, NY; January 23 – May 7.

- 2002: Bio 517A: Topics in Forensic Biology (1 credit), University at Albany, September 5 – December 8.

- 2002: Invited Co-auditor: New York State Department of Health Forensic Laboratory Evaluation Program Audit of Laboratory Corporation of America's Forensic Identity Unit, Research Triangle Park, NC, November 18-20.

- 2003: *NYSP Databank Validation of the TrueAllele™ Automated Data Analysis System.* Presented at the Scientific Working Group on DNA Analysis Methods (SWGDAM).

- 2003: *Topics in Forensic Biology* (Bio 517B: 1 credit, 15 class hours), University at Albany, January 27-May 5.

- 2003: *Forensic Biology Laboratory* (Bio 575: 3 credits, 15 class hours and 60 laboratory hours), NYSP East Campus Training and Research Facility University at Albany, January 22-May 14.

- 2003: *ASCLD/LAB audit of the Arizona Department of Public Safety Laboratory*, auditor of the DNA section, February 2-7.

- Publications:
    - Research Papers:
        - Lawrence, DA; Eastman, A; Weigle, WO. 1978. Murine T cell preparations: radiosensitivity of helper activity. *Cell. Immunol. 36:97.*
        - Regal, JR; Eastman, A; Pickering, RJ. 1980. C5a induced tracheal contraction: a histamine-independent mechanism. *J. Immunol. 124:287.*
        - Eastman, AY; Lawrence, DA. 1982. TNP-modified syngeneic cells enhance immunoregulatory T cell activities similar to allogeneic effects. *J. Immunol. 128:926.*
        - Eastman, AY; Lawrence, DA. 1984. The allogeneic effect: the mechanism of allosuppression by Lyt-1^{+}, Ia^{-}, T cells. *J. Immunol. 133:1155.*
        - Eastman, AY; Lawrence, DA. 1985. 2-Mercaptoethanol dependent, LPS-responsive B cells in Nylon wool fractionated spleen cell preparations. *Scand. J. Immunol. 21:35.*
        - Eastman, AY; O'Neill, A; Reid, K; Garberi, J-C; Karl, M; Flaherty, L. 1986. Extensive deletions in the Q subregion of the murine major histocompatibility complex. *Immunogenetics 24:368.*
        - Chapes, SK; Eastman, AY; O'Neill, A; Flaherty, LA; Gooding, LR. 1987. Macrophage-resistant murine SV40 tumors express type-specific retroviral gp70. *J. Virology 61:928.*
        - Horner, SA; Fisher, HA; Barada, JH; Eastman, AY; Migliozzi, J; Ross, J. 1990. Verrucous carcinoma of the bladder. *J. Urology 145:1261.*
        - Kim, DN; Schmee, J; Lee, CS; Eastman, AY; Ross, JS; Thomas, WA. 1991. Effects of fish oil and corn oil supplements on hyperlipidemic diet induced atherogenesis in swine. *Atherosclerosis 89: 191.*
        - Weinberg, E; Hoisington, S; Eastman, AY; Rice, DK; Malfetano, J; Ross, JS. 1993. Uterine cervical lymphoepithelial-like carcinoma. Absence of Epstein-Barr virus genomes. *Amer. J. Clin. Path. 99:195.*
        - Remick, SC; McSharry, JJ; Wolf, BC; Blanchard, CG; Eastman, AY; Wagner, H; Portuese, E; Wighton, T; Powell, D; Pearce, T; Horton, J; Ruckdeschel, JC. 1993. Novel oral combination chemotherapy in the treatment of intermediate and high-grade AIDS-related non-Hodgkin's lymphoma. *J. Clin. Oncology 11:1691.*
        - Kim, DN; Schmee, J; Baker, JE; Lunden, GM; Sheehan, CE; Lee, CS; Eastman, AY; Solis, O; Ross, JS; Thomas, WA. 1993. Dietary fish oil reduces microthrombi over atherosclerotic lesions in hyperlipidemic swine even in the absence of plasma cholesterol reduction. *Experimental and Molecular Pathology 59: 122.*
        - Ambros, RA; Vigna, PA; Figge, J; Kaliakury, B; Mastrangelo, A; Eastman, AY; Malfetano, J; Figge, HL; Ross, JS. 1994. Observations on tumor and metastatic suppressor gene status in endrometrial carcinoma with particular emphasis on p53. *Cancer 73: 1686 – 1692.*

NEW YORK STATE POLICE
FORENSIC INVESTIGATION CENTER
STATEMENT OF QUALIFICATIONS

- Kim, DN; Eastman, AY; Baker, JE; Mastrangelo, AM; Sethi, S; Schmee, J; Thomas, WA. 1995. Fish oil, atherogenesis and thrombogenesis. *Ann. NY Acad. Sci. 748: 74-481.*

- Ross, JS; Eastman, AY; Mastrangelo, A; Baker, JE; Kim, DN. 1995. Effects of nonsteroidal anti-inflammatory drugs on monocyte-endothelial cell interaction. *Ann. NY Acad. Sci. 748: 559 – 561.*

- Wharton, J; Novak, C; Eastman, AY; Burkart, PT; Wilner, GD. 1995. Management considerations in severe symptomatic idiopathic cold agglutinin disease: Adjunct role of apheresis. *J. Clin. Apheresis 89: 21-24.*

- Slominski, A; Ermak, G; Hwang, J; Mazurkiewicz, J; Eastman, AY. 1995. The expression of proopiomelanocortin (POMC) and of corticotropin releasing hormone receptor (CRH-R) genes in mammalian skin. *Biochimica Biophysica Acta 1289: 247 – 251.*

- Sethi, A; Eastman, AY; Eaton, J. 1996. Inhibition of phagocyte: endothelial interactions by oxidized fatty acids: a natural anti-inflammatory mechanism? *J. Lab. Clin. Med. 128: 27-38.*

- Mastrangelo, A; Eastman, AY; Jeitner, TM. 1996. Inhibition of THP-1 cell attachment to endothelial cells by cromolyn. *Inflammation Research 43: 231-237.*

- Bruce Budowle[1], Arni Masibay[11], Stacey J. Anderson[12], Charles Barna[7], Lisa Biega[9], Susanne Brenneke[8], Barry L. Brown[1], Jill Cramer[14], Barry Duceman[9], **Allison Eastman**[9], Gretchen A. DeGroot[13], Derek Douglas[3], Jennifer Hamil[5], Daniel J. Hasse[13], Dirk W. Janssen[13], Timothy D. Kupferschmid[5], Stephen LaBonne[9], Terri Lawton[7], Christine Lemire[6], Barbara Llewellyn[3], Tamyra Moretti[2], Jennifer Neves[6], Chris Palaski[10], Sidney Schueler[4], Joanne Sgueglia[6], Cynthia Sprecher[11], Chris Tomsey[10], Don Yet[7], Jeff Zachetti[10]. 2001. STR primer concordance data: validation studies. *Forensic Science International:* 3190: 1-8.

- Eastman, AY; Morris, S; Duceman, B. Forensic validation of a rapid screening test for the presence of Prostate Specific Antigen (in preparation).

- Eastman, AY; McKiernan, L; Hart, B; Zevotek, M; Maura, M; Duceman, B. Forensic validation for a rapid screening test for the presence of human hemoglobin (in preparation).

- Forensic science curricula for the undergraduate. *Handbook for Forensic Training and Education.* National Institute of Justice and the University of West Virginia (in preparation).

- Abstracts

  - Eastman, Ay; Lawrence, DA. 1977. Radiosensitivity of the helper activity induced by T cell preparations. *Fed. Proc. 336:1194.*

  - Eastman, AY; Lawrence, DA. 1978. Radiation modulation of the helper activity of allogenic T cell subpopulations. *Fed. Proc.37:1572.*

  - Eastman, AY; Lawrence, DA. 1981. Induction of suppressor and helper activity by hapten-modified syngeneic cells. *In: "Immunobiology of the Major Histocompatibility Complex:* Seventh International Convocation on Immunology" (MD Zaleski, CJ Abeyounis, K Kano, eds.), S Karger Publishers, Inc., New York.

  - Eastman, Ay; O'Neill, A; Flaherty, L. 1986. The evolution of the Q subregion. Presented in the MHC workshop at the *Sixth International Congress of Immunology,* Toronto, Canada, July 6-11.

  - Kim, DN; Schmee, J; Lee, CS; Eastman, AY; Ross, JS; Thomas, WA. 1991. Fish oils reduce platelet-monocyte-endothelial cell interactions over lesions of coronary arteries in swine. *Arteriosclerosis Council Abstracts.*

  - Stellrecht, KA; McSharry, JJ; Venezia, RA; Eastman, AY. 1992. Quantitation of HIV DNA and RNA: improved standardization. *American Society for Virology, 11th Annual Meeting,* Cornell University, Ithaca, NY; July 11-15.

  - Eastman, AY; Baker, J; Kim, Dn. 1993. Eicosapentaenoic acid (EPA) reduces adhesion of monocytes (MO) to lipopolysaccharide (LPS)-induced endothelial celle (EC) *in vitro. J. Immunol. 150:140A.*

  - Stellrecht, KA; Malangia, A; Eastman, AY; Lawrence, DA; Venezia, RA. 1993. Effects of HIV-1 on alveolar macrophage function. *American Society for Virology, 12th Annual Meeting,* University of California, Davis: July 10-14.

  - Burkart, P; Eastman, AY; Lorch, C; Harrison, B; Wolf, B; Ross, J. 1993. Chronic myelogenous leukemia with an unusual variant Philadelphia chromosome, t(16;22)(p13;q11) and chloroma. XII[th] Meeting of the *International Society of Haematology,* Vienna, Austria: August 15-21.

  - Wharton, J; Novak, C; Eastman, AY; Burkart, P; Wilner, GD. 1994. Plasmapheresis in refractory cold agglutinin disease. *World Apheresis Association, 5th International Congress,* March 1-12. Presented at Hematology Symposium.

  - Sethi, S and Eastman, AY. 1994. Eicosapentaenoic acid modulates the expression of vascular endothelial cell adhesion molecules. *27th Annual Lofland Conference on Arterial Wall Metabolism,* Omni Sagamore Resort, Bolton Landing, NY; May 5-7.

  - Sethi, S; Kim, DN; Eastman, AY. 1994. Oxidation enhances the inhibitory effect of eicosapentaenoic acid on the expression of adhesion molecules by vascular endothelial cells. *Circulation 90:I-31.*

- Lorch, CA; Burkart, PT; Eastman, AY; Harrison, BA. 1994. Chronic myelogenous leukemia (CML) with variant Philadelphia chromosome t(16;22)(p13;q11)-cytogenetic and molecular studies. *American Society of Human Genetics.* October 1994, Montreal, Canada.

- Sethi, S; Eastman, AY. Oxidized, polyunsaturated fatty acids as inhibitors of leukocyte-endothelial cell adhesion. *Federation of the American Society of Experimental Biologists,* Atlanta, GA; April 1995.

- Mastrangelo, A; Eastman, AY. Cromolyn reduces THP-1 attachment to endothelial cells. *Federation of the American Society of Experimental Biologists,* Atlanta, GA; April 1995.

- Sethi, S; Eastman, AY; Eaton, JW. Oxidized fatty acids inhibit phagocyte:endothelial interactions: a natural anti-inflammatory mechanism? *American Heart Association, 68th Scientific Sessions,* Anaheim, California: November 13-16, 1995.

- Davies, JMS; Kremer, JM; Furst, DE; Eastman, AY. Lymphomatous changes during methotrexate therapy. *American College of Rheumatology National Scientific Meeting,* October 22-26, San Francisco, California.

- Eastman, AY; Portzer, RM, Duceman, BD. 1996. Validation of PCR DNA amplification services at the New York State Police Forensic Investigation Center. *Northeastern Association of Forensic Scientists, 22nd Annual Meeting,* Pocono Manor, PA. Oral Presentation.

- Eastman, AY; Portzer, RM; Duceman, BW. 1997. Forensic PCR DNA Typing at the New York State Police Forensic Investigation Center: First Six Months Experience. *Northeastern Association of Forensic Scientists, 23rd Annual Meeting,* White Plains, NY. Oral Presentation.

- Morris, S; Eastman, AY. 1998. Forensic validation of two rapid screening tests for Prostate Specific Antigen. *Northeastern Association of Forensic Scientists, 24th Annual Meeting,* Newport, Rhode Island. Poster.

- Eastman, AY; Morris, S; Simich, J; Duceman, B. 1999. Forensic validation of rapid screening tests for Prostate Specific Antigen. *Proceeding of the American Academy of Forensic Sciences 5:46.*

- Morris, S; Biss, T; Eastman, AY. 1999. Forensic validation of rapid screening tests for Prostate Specific Antigen (PSA): Supplemental studies. *Northeastern Association of Forensic Scientists, 25th Anniversary Meeting,* October 14-16.

4.0 FACILITIES

4.1 Laboratory Security

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

4.1.1 Forensic Biology Security

When not under the direct control of Forensic Biology personnel, evidence will be secured either by closing and locking the Forensic Biology door or by its return to secure storage (one of the locked evidence refrigerators/freezers/file cabinets or the analyst's personal evidence cabinet). Only Forensic Biology Personnel will have access to the locked storage. Persons having official business will be allowed access to Forensic Biology only when accompanied by program personnel. Other ISP forensic laboratory visitors will not be allowed in the Forensic Biology laboratory section.

4.1.2 CODIS Security

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

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

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

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

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

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

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

4.1.2.7 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.

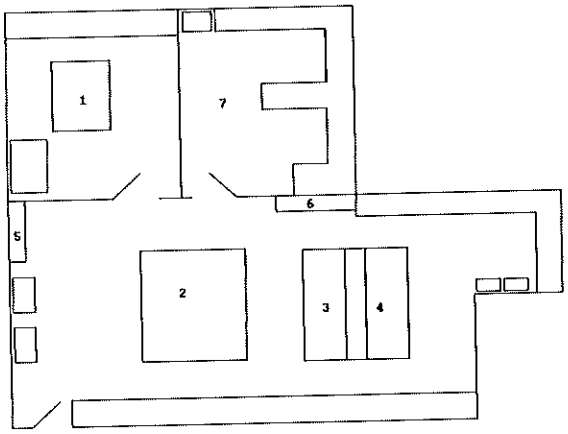
4.1.2.8 CODIS sample information is released only in accordance with 19-5514 of the Idaho DNA Database Act of 1996, and the Privacy Act Notice in Appendix E of NDIS procedures.

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



4.3 Laboratory Cleaning and Decontamination

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

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

4.3.2 All **small tools/instruments** (i.e. forceps, scissors, etc.) will be cleaned/rinsed with ethanol or germicidal instrument cleaner prior to use and between samples.

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

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

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

4.3.6 All work surfaces in the **amplification/post-amp room** are to be cleaned with 10% bleach or Dispatch solution before and after analysis and as part of the monthly QC procedure. Clean white paper and/or a KayDry is to be placed on the benchtop prior to use. Additionally, as part of the monthly QC procedure, the following are to be conducted: the exterior surfaces of the genetic analyzers wiped down with ethanol or Dispatch solution, top of the refrigerator/freezer and surface underneath each genetic analyzer wiped down/dusted, and floor mopped.

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 Quality/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. Written and/or verbal notification will be given to the prosecuting attorney informing him/her of possible consumption and requesting defense counsel be notified of the situation. Before testing will commence, an allowance 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 will be made. An acceptable expert is a **scientist** with **'hands on'**, **forensic** **experience** in both the technology used in the analyses, and the corresponding data interpretation. Additionally, a letter from the prosecuting attorney must be received by the laboratory indicating whether or not the sample may be consumed.

5.2.3 Amplified Product

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

6.0 VALIDATION

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

7.0 CHEMICALS/REAGENTS

7.1 COMMERCIALLY PURCHASED CHEMICALS

7.1.1 Biology Personnel should consult the 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.

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

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

7.2 REAGENTS PREPARED IN-HOUSE

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

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

7.3 CRITICAL REAGENTS

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

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)

8.0 EQUIPMENT CALIBRATION AND MAINTENANCE

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

8.1 BIOLOGY EQUIPMENT/INSTRUMENTATION

8.1.1 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, serial number, location, estimated life expectancy, anticipated replacement date, actual replacement date and a comments section.

8.1.2 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).

8.1.3 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.

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

8.1.5 The SCHEDULE of QC Checks is as follows:

WEEKLY (Form 404-QC)

Nanopure 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
Eye Wash Station Check
Lab Cleaned
Autoclave Clean and Check Sterilization
Artel PCS2 Calibration Check

QUARTERLY

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

ANNUALLY (Form 402-QC)

Pipette Calibration Check (see Artel user's manual for procedure)
Thermalcycler Verification Kit Calibration Check (outside vendor)
Biological and Chemical Hoods Test (outside vendor)
Digital Temperature Recording Devices Calibration Check (outside vendor)
ABI PRISM™ 310 Genetic Analyzers Preventative Maintenance (outside vendor)
Microscope Cleaning/Preventative maintenance (outside vendor)
Centrifuge Calibration Check (outside vendor)

In addition to the above schedule, personnel should check appropriate parameter function on all instrumentation with each use (including calibration of the pH meter at the time of use), perform MacIntosh HD Optimization as needed, and run a matrix for the ABI PRISM™ 310 Genetic Analyzers as needed or following CCD camera and/or laser replacement/adjustment. 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). Data for each new matrix will be filed in the instrument QC binder (see BI-210).

9.0 PROFICIENCY TESTING

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

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

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

10.0 CORRECTIVE ACTION

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

11.0 FILE DOCUMENTATION AND REPORTS

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

11.1 CASE NOTES

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

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

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

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

11.1.5 Whenever feasible, every attempt should be made to gain entry into the evidence without breaking the original

seals. Any seal altered or created by a scientist will bear their initials and date across the seal.

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

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

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

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

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

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

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

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

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

11.1.10 If a form is used for more than one case, a copy of the 'completed' form should be made for any additional case files. Each copy should contain a reference regarding the

location (case file) of the original document. For each file, the associated case should be listed and case data highlighted. In general, subfolders should be organized from front to back as follows: report, chronological case notes/forms (SAECK form where applicable), 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.

11.2 REPORTS

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

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

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

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

RESULTS OF EXAMINATION

Statements (see below) regarding evidence exam, results and conclusions. The order of statements should be, inasmuch as possible: 1) positive statements (detection of body fluid), 2) inconclusive statements, 3) negative statements and 4) statements regarding (i.e. a list of) items not examined.

Disposition of Evidence

Statements (See below) regarding evidence retention and return.

Evidence Description

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

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

Semen Results/Conclusions Statements:

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

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

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

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

Blood Results/Conclusion Statements:

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

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

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

Saliva Results/Conclusions Statements:

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

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

Urine Results/Conclusions Statements:

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

Feces Results/Conclusions Statements:

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

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

If additional testing is desired, please contact the laboratory.

The following samples (or the samples listed below) have been forwarded for DNA analysis: [list items and include known bloodstains from "name" (Item#)]. Results will follow in a separate report.

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

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

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

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

Profile Match Statement [meeting the 'source attribution' criterion (estimated frequency in population of ≤ 1 in $1.6x10^{10}$)]:

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

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

[1]This conclusion is based upon the following: 1) a genetic match at the gender identity locus, Amelogenin, in addition to the following "number" polymorphic STR loci listed below that have an expected population frequency of less than 1 in "actual (most conservative of the population groups calculated) frequency estimate", 2) a statistical frequency exceeding the source attribution criterion (for $N=1.6x10^7$, $\alpha=0.01$; *Forensic Science Communications 2(3)July 2000,* and 3) that "name" does not have a genetically identical twin.

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.

Profile match Statement [not meeting the 'source attribution' criterion (estimated frequency in population of greater than 1 in $1.6x10^{10}$)]:

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

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

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

Mixture Statements:

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

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

Exclusionary Statement:

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

No DNA Profile Obtained Statement:

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

Loci Examined Statement (typically included in footnote):

Loci Examined: (or Loci examined include some or all of the

following) D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, /S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, and FGA.

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

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

Evidence Disposition Section Statements:

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

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

Evidence Description Section Examples:

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

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

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

A tape-sealed DNA packet containing the following items:

Item #) "description"
Item 3) "description"

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

12.0 REVIEW

Laboratory 'casework review' is addressed in the ISP Forensic Services Quality/Procedure Manual. See also, forms 214-BI and 306-BI in this manual.

12.1 BIOLOGY CASEWORK REVIEW

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

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

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

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

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

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

12.2 TESTIMONY REVIEW

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

13.0 SAFETY

Laboratory safety practices are addressed in the ISP Forensic Services Safety Manual. In Forensic Biology, personnel are introduced to these practices in Module 1 of the ISP Forensic Biology Training Manual. In addition, forms 406-QC and 408B-QC (Section 7 of this manual) address the monitoring of safety devices, the chemical eye-wash and shower, respectively.

14.0 AUDITS

Quality audits are delineated in the ISP Forensic Services Quality/Procedure 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 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.

15.0 Practices and Analytical Procedures

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

MBI≡Schemes, generally encompassing many procedures.

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

BI≡Analytical Procedures or Individual Processes

BI-100 PROCESSING LIQUID BLOOD
BI-102 DNA PACKETS
BI-104 PHENOLPHTHALEIN TEST FOR BLOOD
BI-105 O-TOLIDINE TEST FOR BLOOD
BI-106 HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST
BI-108 SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION
BI-110 BIOLOGICAL SCREENING: USE OF ALTERNATE LIGHT SOURCE
BI-114 BRENTAMINE TEST FOR ACID PHOSPHATASE
BI-116 SAMPLE EXTRACTION FOR SEMEN IDENTIFICATION
BI-118 SEMEN IDENTIFICATION: MICROSCOPIC EXAMINATION
BI-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-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 (KASTLE-MEYER)
102-BI HYDROGEN PEROXIDE 3% (v/v)
103-BI O-TOLIDINE REAGENT
104-BI AMMONIUM HYDROXIDE (~3%)
108-BI OUCHTERLONY DESTAIN
110-BI OUCHTERLONY STAIN
114-BI 10X BRENTAMINE (SODIUM ACETATE) BUFFER
116-BI BRENTAMINE SOLUTION A
118-BI BRENTAMINE SOLUTION B
120-BI SALINE (0.85% NaCℓ)
124 BI 1X PHOSPHATE BUFFERED SALINE (PBS)
126-BI XMAS TREE STAIN SOLUTION A (KERNECHTROT SOLUTION)
128-BI XMAS TREE STAIN SOLUTION B (PICROINDIGOCARMINE SOLUTION)
132-BI AMYLASE DIFFUSION BUFFER (pH6.9)
134-BI AMYLASE IODINE REAGENT
138-BI MERCURIC CHLORIDE 10% (w/v)
140-BI ZINC CHLORIDE 10% (w/v)
201-BI 1M TRIS-HCℓ BUFFER pH7.5
203-BI 1M TRIS-HCℓ BUFFER pH8
205-BI ETHYLENEDIAMINE TETRAACETIC ACID (EDTA) 0.5M
207-BI STAIN EXTRACTION BUFFER pH8
211-BI PROTEINASE K (20 mg/mℓ)
222-BI 1M SODIUM ACETATE pH5.2
223-BI DTT (1M)
225-BI CHELEX REAGENT 5%
229-BI PCR-TE (TE^{-4}) BUFFER (10mM TRIS-HCℓ, 0.1M EDTA)
231-BI NaOH 5N
233-BI SODIUM CHLORIDE (NaCℓ) 5M
240-BI QUANTIBLOT PRE-WETTING SOLUTION (QPW)
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%
101-BI BIOLOGY SCREENING SUMMARY
200-BI DNA EXTRACTION WORKSHEET
202-BI DIFFERENTIAL DNA EXTRACTION WORKSHEET
204-BI QUANTIBLOT 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

MBI-100	
11/9/04	11-23-04
CRN	RJR

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. 73-138.

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

2.0 SCOPE:

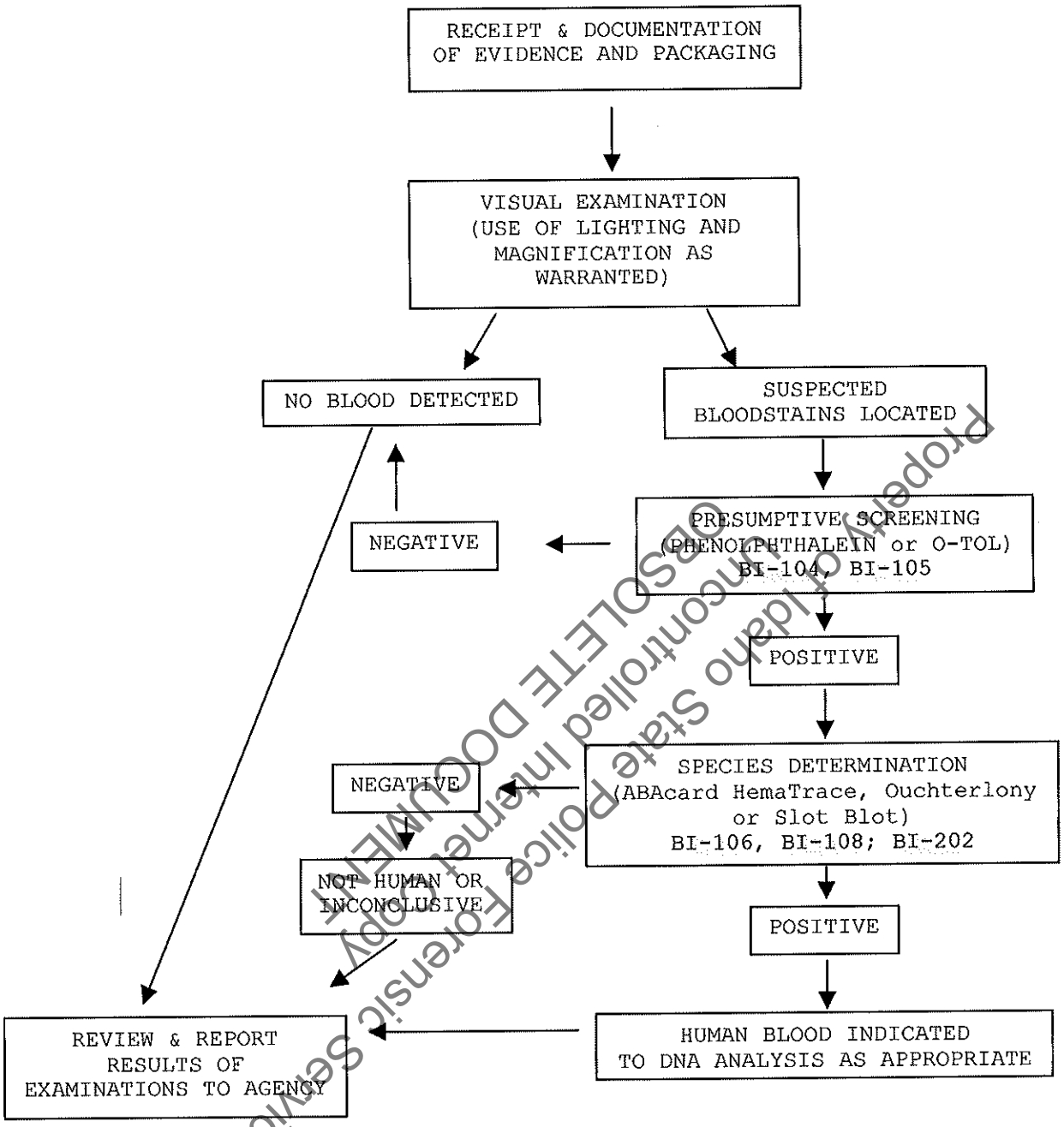
To provide uniform processing of evidentiary material for the presence of blood.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

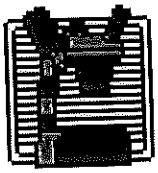
See Flow Chart on following page.



5.0 COMMENTS:

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

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



BI-100	
11/9/04	11-23-04
CRN	[illegible]

PROCESSING LIQUID BLOOD

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

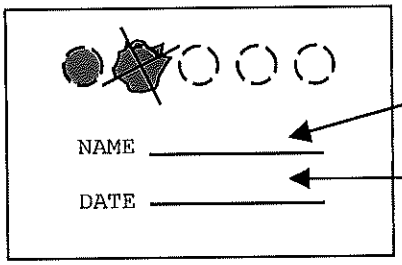
Schleicher & Schuell: Blood Collect Card(s) #903
Coin Envelopes
Disposable Transfer pipet or 1 mℓ pipet with sterile tip

4.0 PROCEDURE:

4.1 Label stain card with a minimum of Case Number, 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, or M20011234/1A)

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 (~3⅛" x 5½"). Seal envelope with evidence tape on flap and label with initials across seal. Label front of coin envelope with Case Number and Item Number minimally.

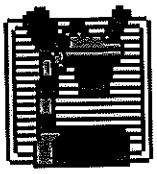
4.6 Make Case DNA Packet (See BI-102) and place bloodstain sample inside.

5.0 COMMENTS:

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

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

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



BI-102	
11/9/04	11-23-04
CRH	RDS

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 (~3⅛" x 5½", and other sizes as needed)
DNA Packet Envelope (~6½" x 9¼" manila envelope)

4.0 PROCEDURE:

4.1 Cuttings/swabs containing previously identified biological evidence, as well as known reference bloodstain cards should be packaged in separate coin envelopes. 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.

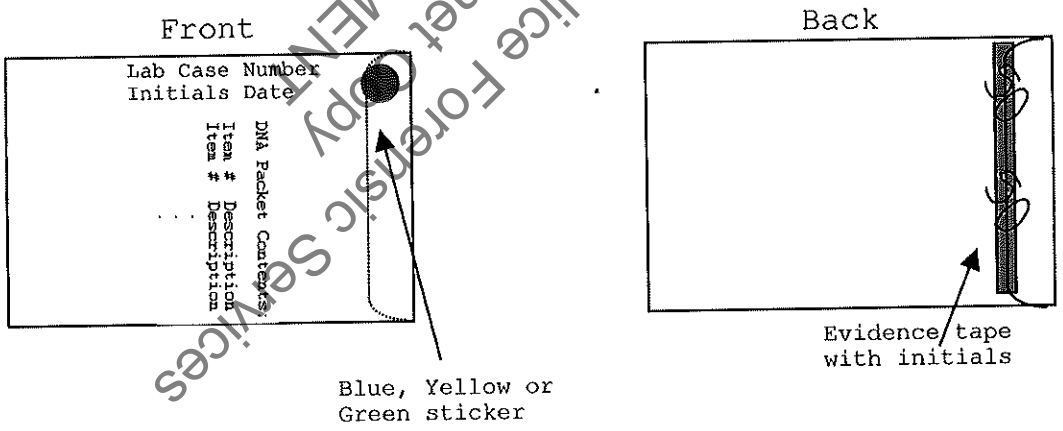
4.2 All sealed envelopes will be placed inside a larger manila envelope (DNA Packet Envelope) and labeled as below.

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 PES and entered as an additional item of evidence to allow for tracking in the ETS. The storage location will have a barcode.

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



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

5.0 COMMENTS:

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

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

5.3 On **RARE** occasions when it is deemed necessary to have more stains collected in a given case than will fit into a single DNA Packet Envelope, the DNA Packets should still be entered into ETS as a single evidence item but labeled "1 of X, 2 of X ...X of X".

BI-104	
11/9/04	11-23-04
CRH	RDE

PHENOLPHTHALEIN TEST FOR BLOOD

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

Phenolphthalein Working Solution
3% Hydrogen Peroxide
Cotton Swabs or Filter Paper

4.0 PROCEDURE:

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

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

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

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

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

5.0 COMMENTS:

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

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

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

BI-105	
11/9/04	11-23-04
CRN	

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 H_2O) control samples are processed, prior to testing any forensic samples (on the day of testing), to ensure the working stock reagents are functioning properly.

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

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

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

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

5.0 COMMENTS:

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

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

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

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

BI-106	
11/9/04	11-23-04
CRA	KRP

HUMAN BLOOD IDENTIFICATION USING ABACARD® HEMATRACE® TEST

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABACard® Hematrace® Test Kit

4.0 PROCEDURE:

4.1 Label extraction tubes for identification.

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

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

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

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

5.0 COMMENTS:

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

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

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

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

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

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

BI-108	
11/9/04	11-23-04
CRN	[illegible]

SPECIES IDENTIFICATION: OUCHTERLONY DOUBLE DIFFUSION

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

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

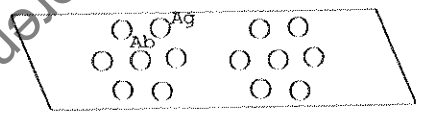
4.0 PROCEDURE:

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

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

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

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



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

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

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

4.7 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 dH_2O for ~5 minutes; dampen two pieces of filter paper with dH_2O 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.

4.7.5 Rinse with dH_2O and allow to dry.

5.0 COMMENTS:

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

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

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

MBI-102	
11/9/04	12-23-04
CRP	RLC

EXAMINATION OF EVIDENCE FOR SEMEN

1.0 BACKGROUND:

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

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

2.0 SCOPE:

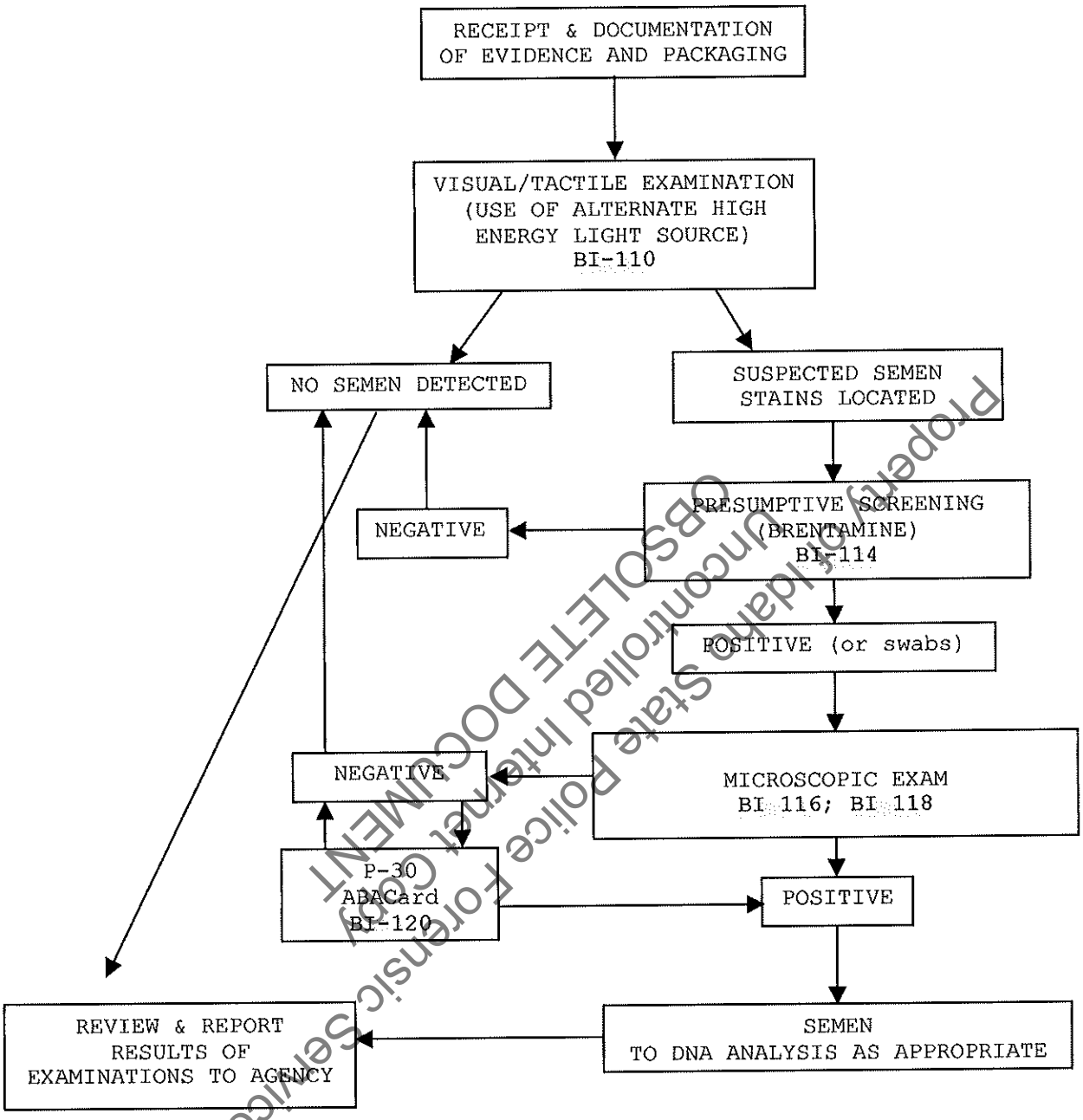
To provide uniform processing of evidentiary material for the presence of semen.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

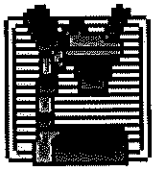
See Flow Chart on following page.



5.0 COMMENTS:

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

5.2 A P-30 test need not be performed on vaginal swabs which yielded a positive microscopic exam.



BI-110	
11/9/04	1-23-04
CRN	RDL

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

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

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

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

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.

BI-114	
11/9/04	11-23-04
CRA	RHG

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. This solution should be prepared fresh each day it is used.

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

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

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

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

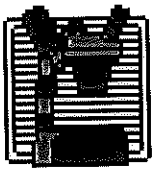
5.0 COMMENTS:

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

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

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

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



BI-116	
11/9/04	11-24-04
CRA	N/A

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

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

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

5.0 COMMENTS:

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

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

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

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

BI-118	
11/9/04	11-23-04
CRH	RDS

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

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

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

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

BI-120	
11/9/04	1/-2424
CRH	ABA

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

1.0 BACKGROUND:

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

OneStep ABAcard® p30 Test Kit

4.0 PROCEDURE:

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

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

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

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

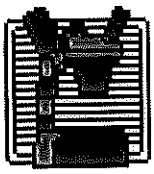
5.0 COMMENTS:

5.1 Samples must be at room temperature for the test.

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

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

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



MBI-104	
11/9/04	11-23-04
CRN	

EXAMINATION OF EVIDENCE FOR BODY FLUIDS

1.0 BACKGROUND:

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

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

2.0 SCOPE:

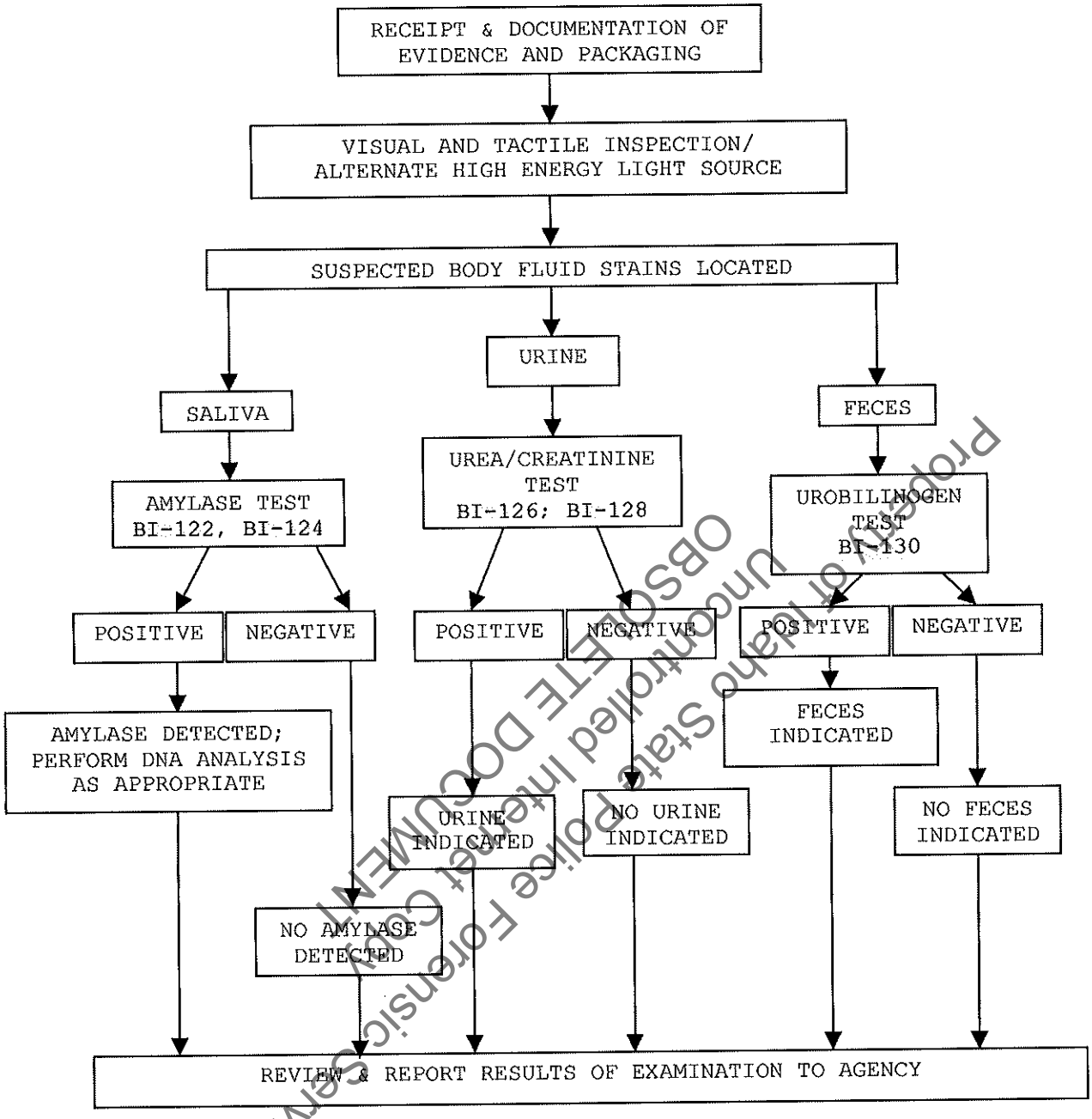
To provide uniform processing of evidentiary material for the presence of saliva, urine or feces.

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

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

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

BI-122	
11/9/04	11-23-04
CRM	MDS

AMYLASE TEST (PHADEBAS)

1.0 BACKGROUND:

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

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

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

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

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

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

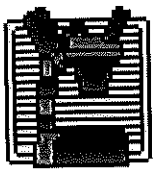
4.4 Examine tubes and record the color of the supernatant. The intensity of the blue color, if present, may be graded as light, medium, dark, or 1^+-4^+. For reporting, see **5.1**.

5.0 COMMENTS:

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

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

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



BI-124	
11/9/04	11-24-04
CRN	

AMYLASE TEST (STARCH IODIDE)

1.0 BACKGROUND:

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

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

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

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

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

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

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

4.4 Mark petri dish for orientation and document sample placement.

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

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

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

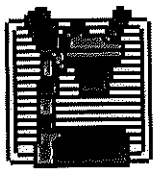
5.0 COMMENTS:

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

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

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

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



BI-126	
11/9/04	11-23-04
CRN	RWG

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

4.2 Add 3-4 drops of dH_20 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 (dH_2O blank) and a substrate control where appropriate and available.

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

BI-128	
11/9/04	11-23-04
CPP	MDS

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

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

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

4.5 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 (known urine stain) and negative (dH_2O blank) and a substrate control where appropriate and available.

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

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

5.3 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	
11/9/04	11-23-04
CRN	RDL

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

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

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

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

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

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

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

5.0 COMMENTS:

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

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

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

MBI-200	
11/9/04	11-24-04
CRN	KKE

INDIVIDUALIZATION OF DNA SOURCES BY STR ANALYSIS

1.0 BACKGROUND:

Once a DNA source has been detected, and identified as to 'source type' where applicable and feasible, it is generally important to attribute the DNA sample to a particular individual inasmuch as possible. Current DNA technology, in the analysis of STR loci, offers individualization potential.

2.0 SCOPE:

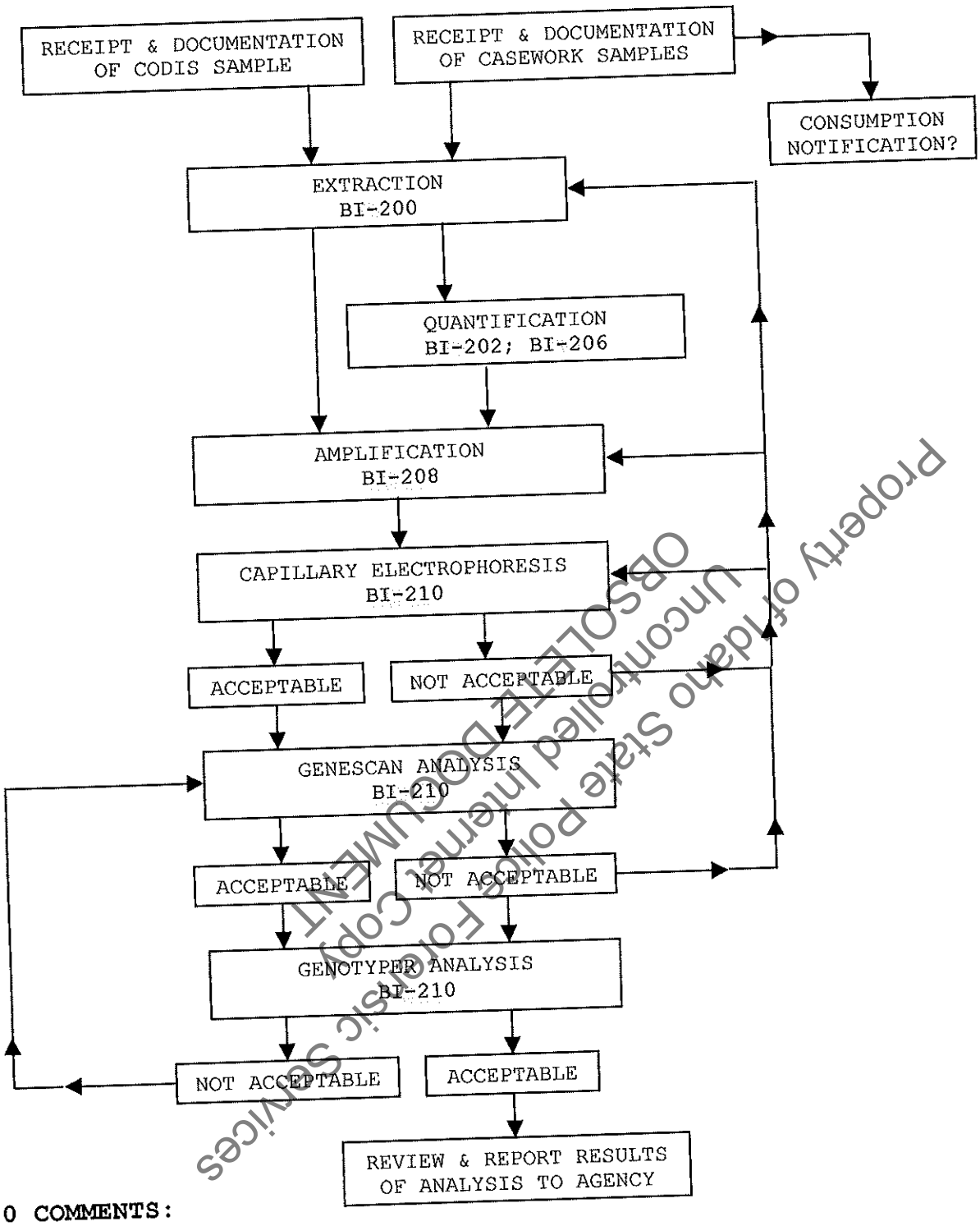
To provide uniform processing of DNA samples to achieve high quality data and consistent interpretation.

3.0 EQUIPMENT/REAGENTS:

As listed in individual analytical procedures.

4.0 PROCEDURE:

See Flow Chart on following page.



5.0 COMMENTS:

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

BI-200	
11/9/04	11-23-04
CRA	RDL

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
56/65°C heat block/oven
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)
Proteinase K (ProK, 20 mg/mL)
1M Dithiothreitol (DTT)
Phenol/Chloroform/Isoamyl Alcohol (PCIAA, 25:24:1)
Ethanol (EtOH)
Phosphate Buffered Saline (PBS)
Ethyl Ether
Xylene
10% SDS
Liquid Nitrogen
Dry Ice
FTA Purification Reagent
Chelex Reagent

5.0 DNA EXTRACTION PROCEDURES:

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

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

Caution: See Comments 1 and 4.

5.1 BLOOD/SALIVA/NON-SEMEN (TISSUE, EPITHELIAL CELLS) SAMPLES:

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

5.1.1a Envelope Flaps/Stamps: Presoak the envelope flap/stamp cutting (steam may be used to loosen the seal prior to extraction) in 1.0mℓ of sterile water at 4°C for a minimum of 5 hours (may be left overnight). Remove the substrate by piggyback/spin basket centrifugation. Remove all but 50µℓ of the supernatant and discard it. Proceed to **5.1.2** with the remaining pellet.

5.1.1b Optional (see **Comments 3**): Presoak bloodstains using 1 mℓ 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:

500µℓ SEB
15µℓ Pro K

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

5.1.3a Optional: For most stains the cuttings/substrate will not interfere with organic extraction and need not be removed prior to proceeding to **5.1.4.** Larger cuttings/samples can be removed by piggyback/spin basket centrifugation at low speed (3,000 - 5,000 rpm) for 3-5 minutes. Proceed to **5.1.4.**

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

5.1.5 If the aqueous phase is clear, proceed to **6.0.** If it is not clear (e.g. cloudy or large or 'dirty' interface), transfer the aqueous layer to a fresh sterile 1.5mℓ tube. Repeat **5.1.4** 1-2 times until the interface is clean and aqueous phase is clear. Proceed to **6.0.**

5.2 DIFFERENTIAL EXTRACTION OF SEMEN-CONTAINING SAMPLES:

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

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

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

5.2.2a Optional: Resuspend the pellet in the remaining 50µℓ and place 3-5µℓ on a slide for microscopic evaluation (See BI-118). The substrate may be discarded if the pellet contains a sufficient number of spermatozoa; however, it may be desirable to add the substrate back to increase the total amount of DNA in the sample.

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

500µℓ SEB
15µℓ Pro K

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

5.2.5 Mark a new sterile 1.5mℓ tube. Remove substrate by using piggyback/spin basket centrifugation. A final centifugation on high speed for ≥1 minute should be performed to further solidify the pellet.

5.2.6 Remove all but ~50µℓ of the supernatant, taking care not to disrupt the cell pellet in the bottom of the tube. Transfer this supernatant (epithelial cell fraction) to the new, marked sterile tube and store at 4°C or proceed directly to **5.2.11.**

5.2.6a Optional: The purpose of a differential extraction is, typically, to obtain a sperm fraction that is void of any epithelial contribution. In instances in which there is an overwhelming proportion of epithelial cells to sperm that appear intact microscopically, steps **5.2.3-5.2.4** may, at the scientist's discretion, be repeated 1-2 times prior to proceeding to **5.2.7.** These additional supernatants do not need to be retained.

5.2.7 Wash the sperm pellet as follows: Resuspend the pellet in 500-1000µℓ PBS or SEB by vortexing briefly. Spin in a microcentrifuge for ~5 minutes at maximum speed (>10,000rpm). Remove all but ~50µℓ of the supernatant and discard it.

5.2.8 Repeat **5.2.7** 1-5 more time(s). In instances of low sperm amounts, additional washes are recommended. The final wash performed is to be done using 500-1000µℓ sterile deionized water.

5.2.8a Optional: Resuspend the pellet in the remaining 50µℓ and place 3-5µℓ on a slide for microscopic evaluation (See BI-118). If intact epithelial cells remain, the pellet should be redigested (**5.2.3 - 5.2.8**).

5.2.9 To the remaining sperm pellet solution add:

500µℓ SEB
20µℓ 1M DTT
15µℓ ProK

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

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

5.2.12 If the aqueous phase is clear, proceed to **6.0.** If it is not clear (e.g. cloudy or large or 'dirty' interface), transfer the aqueous layer to a fresh sterile 1.5mℓ tube. Repeat **5.2.11** 1-2 times until the interface is clean and aqueous phase is clear. Proceed to **6.0.**

5.3 EXTRACTION FROM HAIR:

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

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

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

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., microscopic exam, mitochondrial DNA).

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

500µℓ SEB
20µℓ 1M DTT
15µℓ ProK

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

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

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

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

Caution: See Comments 4.

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

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

5.4.3 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.

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

5.4.5 To the tube, add:

500µℓ SEB
15µℓ 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.

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

5.5 EXTRACTION OF DNA FROM AGED BONES:

Caution: See Comments 4.

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

5.5.2 Rinse bone with sterile, deionized water (shake vigorously with a few mℓ's of water in a 15mℓ conical tube).

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

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

toward pouring spout and slowly transfer to a 50mℓ polypropylene tube. Allow the liqN_2 to evaporate completely.

5.5.5 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.

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

5.5.7 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. Proceed to **6.0.** This will need to be accomplished in a stepwise fashion, with consolidation of the final extracts at the end.

5.6 EXTRACTION FROM FTA/CODIS SAMPLES:

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.

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

5.6.1 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).

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

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

5.6.4 Repeat **5.6.2-5.6.3** twice.

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

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

5.6.7 Repeat **5.6.5-5.6.6** twice.

5.6.8 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.

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

5.7 CHELEX EXTRACTION:

Note: Chelex may also be used for clean-up of samples that have already been extracted to remove contaminants/inhibitors as needed. Start with step **5.7.4**.

5.7.1 Place an ~3mm² cutting of a bloodstain, or 3µℓ whole blood into a sterile 1.5mℓ tube and add 1mℓ of sterile deionized water.

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

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

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

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

5.7.6 Vortex at high speed for 5-10 seconds.

5.7.7 Incubate in boiling water for 8 minutes.

5.7.8 Vortex at high speed for 5-10 seconds, followed by centrifugation at high speed (≥ 10,000 rpm) for 2-3 minutes. This extract may be taken directly to slot blot hybridization (see BI-202) for quantification of the DNA.

note: Care must be taken to not disturb the Chelex resin when removing sample for subsequent procedures. After storage and prior to sample removal, repeat step **5.7.8.**

6.0 DNA ISOLATION PROCEDURE:

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

6.1 ISOLATION VIA CENTRICON CONCENTRATOR DEVICE:

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

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

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

6.1.4 Cover the Centricon tube with the retentate cup. Spin in a fixed angle centrifuge at ~2800 rpm for 10-20 minutes. The DNA sample will be concentrated in ~20-40µℓ of TE in the upper Centricon reservoir, while molecules with molecular weights of less than ~100,000 daltons will pass through the filter.

Note: The Centricon units are sensitive to rotor forces. Do not centrifuge above 3000 rpm. Centrifugation time can be increased if the volume does not reduce to ≤40µℓ in the specified time.

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

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

6.1.7 Invert the upper reservoir onto the retentate cup provided with the unit. Centrifuge at ~1800 rpm for 3 minutes to transfer the DNA concentrate into the cup.

6.1.8 Estimate the volume of the concentrate using a pipette to transfer to a labeled sterile 1.5mℓ tube. Proceed to slot blot hybridization (see BI-202).

7.0 REMOVING MATERIAL FROM SLIDES:

7.1 FREEZING:

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

7.1.2 Wearing safety glasses, pry the cover slip off.

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

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

Note: Sperm-containing slides are rinsed with sterile deionized water at this point and a suitable volume (~100µℓ) of stain extraction buffer (see **5.2.3**) may be added directly to the slide. Incubate ~5 minutes at RT and then by pipetting up and down, wash the sample off of the slide and transfer to 1.5mℓ tube. Repeat 3-4 times and proceed to **5.2.3**.

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

7.2 SOAKING IN XYLENE:

7.2.1 Soak the slide in xylene for several hours until the cover slip can be slid or pried from the slide. **Note:** This will likely remove markings from the slide.

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

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

8.0 Comments:

8.1 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).

8.2 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.

8.3 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).

8.4 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.

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

BI-202	
11/9/04	11-23-04
CRA	PDA

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
Pre-Wetting Solution (QPW)
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 7 sterile microfuge tubes A through G.

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

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

4.1.4 Prepare a serial dilution series by mixing and subsequent 20µℓ transfers from tubes A through G. Store standards at -20°C between uses. The dilution series consists of 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng in 5µℓ, respectively. 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 pre-wetting solution for 1-30 minutes at RT.

4.2.3 Prepare the pre-hybridization solution by mixing 100mℓ pre-warmed hybridization solution and 5mℓ of 30% H_2O_2 in a plastic hybridization tray. Place the lid on the tray and keep at 50°C until use.

4.2.4 Prepare the DNA standards, and samples by combining 5µℓ of each standard and 1-5µℓ of each sample with 150µℓ of spotting solution. 1.25ng of previously quantified 9947A may be used as a control if desired.

4.2.5 Moisten the gasket with a minimal amount of pre-wetting solution. 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.

4.2.6 Carefully pipet standards and samples into appropriate slot-blot wells, avoiding bubbles and contact with the membrane. Wells that do not contain a standard or sample are to be filled with 150µℓ of spotting solution.

4.2.7 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: Transfer the membrane to the plastic tray containing the pre-warmed pre-hybridization solution. Place the lid on the tray. Rotate in a 50°C (±1°C) water bath (50-60 rpm) for 15 minutes (±2 minutes). Pour off the solution.

4.3.2 Briefly rinse membrane in a small amount of pre-warmed hybridization solution. Pour off the solution.

4.3.3 Hybridization: Add 30mℓ of hybridization solution to the tray containing the membrane. Tilt the tray to one side and add 20µℓ of QuantiBlot D17Z1 probe. 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.

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

4.3.5 Stringent Wash/Conjugation: Add 30mℓ of the pre-warmed wash solution to the tray containing the membrane. Tilt the tray to one side and add 90µℓ of Enzyme Conjugate: HRP-SA. 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.

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

4.3.7 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.

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

4.4 DETECTION:

4.4.1 Add chemiluminescence reagents (see **5.2**) to a clean plastic tray and mix by swirling briefly. Add the membrane to the solution, covering completely and rotate for 1-5 minutes per manufacturer's instruction.

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 **4.3.3** 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 Mannhein and Amersham Pharmacia Biotech) have two components that need to be mixed, typically in equal volumes (5mℓ each), just prior to use. The reagents should be aliquoted and allowed to come to RT in a dark location approximately 15 minutes prior to use/mixing. Check product insert for information.

5.3 Clean the slot-blot apparatus thoroughly by soaking (e.g., Neutrad or 0.1% SDS).

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-206	
11/9/04	11-23-04
CRW	RDC

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 Using forceps, place the membrane, DNA-side down, directly on the platen of the image station.

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.

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 analyst's 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	
11/9/04	11-23-04
CRD	RDG

STR AMPLIFICATION: PP16

1.0 BACKGROUND:

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

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

GenePrint® PowerPlex™ 16 System Technical Manual

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

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

4.0 PROCEDURE:

4.1 DNA TEMPLATE:

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

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

4.2 AMPLIFICATION SET-UP:

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

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

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

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

Note:

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

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

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

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

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

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

4.2.7 Ensure all of the 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/CODIS) 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/dishwashing room. The container will be placed into a second biohazard bag, sealed 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 ST★R 10X Buffer, this may be eliminated by briefly heating the solution at 37°C prior to mixing.

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

BI-210	
11/9/04	11-23-04
CAS	PDG

STR TYPING: CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS

1.0 BACKGROUND:

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

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

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

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

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

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 when necessary due to performance, or after any instrument maintenance/repair that involves adjustment/replacement of the CCD camera or laser.

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

4.1.2 The ABI PRISM® 310 Collection Software should open upon start-up. In the manual control window, the scientist may use 'temperature Set' to set the heat plate to 60°C so that it will be ready to run. Using File/New/Sample Sheet, create a 48-well Genescan® sample sheet as described in the ABI PRISM® 310 Genetic Analyzer's User's Manual. If there is room on the sample sheet, 'CCD' and 'SEQFILL' 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: **XY99999999-(or /)ZZ...**,
(e.g., VM20010112-1AF or VM20010112/1AF) where:

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

CODIS samples: 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.

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 24.5µℓ of ILS Master Mix (made by adding 0.5µℓ 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 ~0.5µℓ-1µℓ Ladder to 24.5µℓ Master Mix.

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 ≥3 minutes (perform on all sample types - ladders, matrix, controls and samples).

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

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

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

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

4.1.11 After completion of the run, finish filling out the 310 Injection Log (Form 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 "MMDDYY").

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 (see **4.4.2** RFU Threshold) 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 ≤ 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/or 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 if desired. Alternatively, the scientist may 'click' off the label on these peaks and note the presence of bleed-through on the GT electropherograms. Changes to RFU or use of CC matrix, must also be noted on the electropherogram.

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 sample with the word "ladder" in it. If anomalies such as many off ladder alleles appear 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 and as a method for monitoring facility decontamination. In 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	THO1	8,9.3
D21S11	30,30	D18S51	15,19
Penta E	12,13	D5S818	11,11
D13S317	11,11	D7S820	10,11
D16S539	11,12	CSF1PO	10,12
Penta D	12,12	AMELOGENIN	X,X
vWA	17,18	D8S1179	13,13
TPOX	8,8	FGA	23,24

4.4.1.3 The purpose of the **NEGATIVE AMPLIFICATION CONTROL** is to determine if any human DNA contamination occurred in the process of amplification set-up (or beyond that point) and as another method of monitoring facility decontamination. In the Genescan® electropherograms, peaks above threshold should only appear in the CXR (red dye) lane, corresponding to the ILS600 size standard. Electropherograms for the blue, green and yellow dyes should show a relatively flat baseline throughout the range (discounting primer signal, fluorescent 'spikes' or CXR bleed-through). If detectable signal, with characteristic 'peak' shape, is visible in the electropherogram of a negative amplification control and does not disappear upon re-injection, results for all of the samples associated with that amplification will be examined for the presence of the same peak(s). It is possible, since this control is processed last and its tube deliberately left open during the amplification set-up (to demonstrate maximum contamination potential), that it would be the only sample affected.

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

4.4.1.4 The purpose of a **BLIND CONTROL** sample is primarily to assess correct genotyping, however, it does take measure of all of the steps in the analytical process from extraction through allele designation. The blind controls consist of ~3mm^2 cuttings of previously typed bloodstains. Cuttings are prepared in batches and given random numbers. The scientist is not aware of the

genotype of the sample. Source profiles are maintained by the unit supervisor/technical manager and are provided to the reviewer at the time of case review and only for the associated control(s). A blind control must be run with every batch of forensic cases (will generally be extracted with reference samples or non-semen evidence). The reviewing scientist will complete a Blind Control Check Form (Form 212-BI) for verifying correct genotype(s). A copy of this form will be included in each associated case file or CODIS Data Binder. Note: For CODIS offender buccal runs either an organic 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 the analysis threshold (based on data obtained and signal/baseline) will not be interpreted but should be noted as being present in the case notes (eg. on the table of results).

4.4.2.4 Peaks >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 (≥ 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.

4.4.2.6 Multiplex amplification kits are designed so that heterozygous loci in single-source samples generally demonstrate relatively balanced peak heights [typically ≥70% peak height ratio (phr)]. Some samples, although single-source, may at times demonstrate greater imbalance due to degradation, stochastic effects, primer binding site mutations, preferential amplification, etc. Peak height ratios for these loci (<70% phr) should be calculated and noted on the corresponding electropherogram in the case file or CODIS binder.

4.4.3 EXTRA PEAKS (NON-MIXTURES)

4.4.3.1 PCR amplification of STR loci typically produces a minor product peak one core repeat unit shorter than the main allele peak (n-4 for tetranucleotide loci and n-5 for pentanucleotide loci). This minor peak is

referred to as the **stutter** peak. Percent stutter generally increases with allele length and does not change significantly with the quantity of input DNA (peak heights within ~150-4500 RFU). The measurement of percent stutter may be unnaturally high for main peaks that are off-scale or due to problems with matrix performance and can be corrected by diluting (or reamplifying less DNA) the sample and/or applying a new (or CC) matrix. Loci stutter values are listed in Appendix A to assess potential contribution to peaks in stutter positions.

4.4.3.2 Electronic or fluorescent **spikes** are random events that produce generally spike-shaped peaks in most or all dye colors at the same location (equivalent bp size) within a single injection. Peak heights usually vary between dye colors for a given spike. These anomalies are generally not reproducible and will typically be eliminated upon reinjection. If the spike falls within an allelic range that could interfere with either computer analysis or scientist's analysis, the scientist will print the Genescan® 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.

4.4.3.3 Dye "**blobs**" are anomalies that typically occur in the same approximate location in multiple injections and do not always disappear upon reinjection. Blobs generally look like broad or irregular peaks and may occur in a single color or multiple colors at the same approximate location but can vary in height. The blob should be labeled on the electropherogram if it falls within a diagnostic region and is of significant size to potentially interfere with analysis.

4.4.3.4 **Bleed-through** or pull-up peaks are a result of the matrix not correcting for all of the spectral overlap (most common with the PowerPlex 16 kit from yellow into red). These peaks are in the same location (same bp size) as peaks in another color(s) and are easily recognized. The presence of bleed-through should be noted on the corresponding electropherogram in the case file or CODIS binder. If bleed-through occurs in a color other than red, a new matrix or color corrected matrix may be used at the analyst's discretion to correct for the problem.

4.4.3.5 *Taq* Polymerase can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3' ends of double stranded PCR product, resulting in product one base pair longer than the actual target sequence (+A). Amplification parameters include a final extension time, so that the reaction is driven to full A addition (i.e. all product is +A). **Split-peaks** may occur as a result of incomplete A addition and appear as a single allele represented by two peaks one base pair apart (-A and +A). This can occur when the amount of template DNA is too great (overloaded sample). In this instance, *Taq* is unable to add the A nucleotide to the entire amount of product generated in the time allotted. These samples will typically contain off-scale data as well. Split peaks can be alleviated by incubating samples at 60°C for an additional 45 minutes, followed by dilution prior to reinjection. It may be necessary to re-amplify the sample with less template DNA.

4.4.3 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 (i.e. partial profiles); 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 **Biology QA Manual, section 11.2.4** for reporting of statistical frequencies).

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

4.4.4.3 The frequency for a homozygous profile is determined by the equation $f_{(pp)} = p^2+p(1-p)\theta$, where $\theta = 0.01$ except where small isolated populations (e.g., Native Americans) may be relevant, in which case, $\theta=0.03$.

4.4.4.4 For single-source evidentiary samples (or mixtures for which a distinct genotype(s) is discernible) the statistical consideration will be in the form of a **RANDOM MATCH PROBABILITY** (RMP; or inverse probability of inclusion). The RMP is the inverse of the calculated profile frequency (e.g., for $f_{(STR\ profile)} = 2 \times 10^{-14}$, RMP= 1 in 5×10^{13}; See **Biology QA Manual, section 11.2.4** for reporting of statistical frequencies).

4.4.4.5 For mixtures for which distinct genotypes are not discernible, the scientist may elect to use either the **LIKELIHOOD RATIO (LR)** or **PROBABILITY OF EXCLUSION (PE).**

The LR compares the probability of the occurrence of the evidentiary profile under two hypotheses regarding the composition of the profile and is in the form:

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

The larger the **LR**, the more likely H_1 was the true hypothesis (See **Biology QA Manual, section 11.2.4** for reporting of statistical frequencies). For a paternity calculation, this

corresponds to the PI (Paternity Index).

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

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

$P_E = 1-P_I$
Where $P_I = (p_1 + p_2 + p_3 \ldots p_x)^2$ (the square of the sum of the frequencies of all alleles present in the evidentiary sample).

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

$P_{Ecombined} = 1 - [(1- P_{E1})(1- P_{E2})(1- P_{E3})\ldots(1- P_{E15})]$
(See **Biology QA Manual, section 11.2.4** 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 **Biology QA Manual, section 11.2.4** 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., α=0.01; 1-α=99% confidence).

The FBI has selected an upper confidence limit (UCL) of 99% (α=0.01) and an "N" equivalent to the U.S. population (2.6×10^8 pre-2000 census). This is reasonable as the FBI performs casework for jurisdictions all over the country, and this calculation would provide a uniform approach to be used regardless of jurisdiction. For these figures, an RMP of $>3.9 \times 10^{-11}$ (or less than 1 in 2.6×10^{10}) would confer 99% confidence that the evidentiary profile is unique in the population. However, an additional 10-fold conservation factor, as recommended in NRC II, is added to this figure resulting in a frequency of less than 1 in 2.6×10^{11} for the reporting of source attribution.

In Idaho, using 2000 consensus figures, an operational population (N) of 1.6×10^7 has been selected (representing the sum of the populations of Idaho and the six surrounding states: ID=1.3×10^6; MT=0.9×10^6; NV=2.0×10^6; OR=3.4×10^6; UT=2.2×10^6; WA=5.9×10^6 and WY=4.9×10^5). Therefore, an RMP of less than 1 in 1.6×10^{10} (including 10-fold conservation) will define source attribution (at 99% UCL) for analyses performed in this laboratory (See **Biology QA Manual, section 11.2.4** for reporting of statistical frequencies).

BI-301	
11/9/04	11-23-04
CRM	

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 may be mailed to the laboratory separately; in the event that a sample has not been received, the submitting agency should be notified.

4.1.2 Where possible, compare the DNA sample card information to that of the Collection Report Form to ensure accuracy. Data for a sample may be entered in absence of a DNA Collection Report form, however, an IDOC# is necessary for data entry into STiMAS. Contact necessary IDOC personnel or search the corrections website (www.corrections.state.id.us) for a number if one has not been recorded. The DNA Collection Report Form will be retained after 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 (additional criteria may be used if desired):

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	
11/9/04	11-24-04
CRN	PAE

CODIS SAMPLE DATA ENTRY AND UPLOAD

1.0 BACKGROUND:

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

2.0 SCOPE:

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

3.0 EQUIPMENT/REAGENTS:

CODIS Computer Workstation

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

CODIS Training Manual

4.0 PROCEDURE:

4.1 SAMPLE DATA VERIFICATION:

4.1.1 Genotypic data (allele calls) are checked for accuracy and verified during the CODIS/Casework Review process and documented on the appropriate form (Form 306-BI and Form 214-BI, respectively).

4.1.2 Additionally, when 'STR Data Entry' is used to enter individual sample data (generally forensic samples) verification of 1st and 2nd 'reader' is automatically achieved prior to NDIS upload.

4.2 SAMPLE DATA ENTRY IN CODIS:

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

4.2.2 A second 'read' must also be entered for the individual samples through 'STR Data Entry'. This may be done by the analyst entering the sample data, or by another analyst logging on and entering the allelic data in the second read box. If a single individual enters both reads, the entire profile (i.e. all loci) for the first read must be entered prior to entering any data into the second read. A 'check' indicates agreement between readers at individual loci and discrepancies in entry must be rectified before upload to NDIS.

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

4.3 CODIS DATA UPLOAD:

4.3.1 NDIS There are various reasons that some samples present at SDIS should not be uploaded to NDIS

(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-303.

5.0 COMMENTS:

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

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

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

BI-303	
11/9/04	11-23-04
CRN	

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.

BI-310	
11/9/04	11-24-04
CRA	RKA

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 Workstation
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.

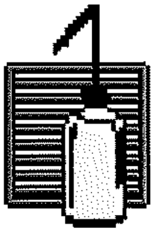
4.2.3 The data for the specified sample will be removed from existing databases (i.e. LDIS and SDIS). After sample removal, a full upload to NDIS will be performed to remove sample at the national level. The sample deletion will be reported (in writing) to the NDIS Custodian and a deleted specimen report 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.

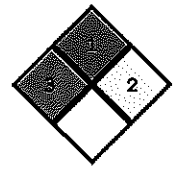
4.3 DOCUMENTATION

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 (KASTLE-MEYER) REAGENT

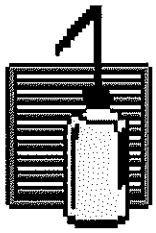
Phenolphthalein	2.0g
KOH	20.0g
Zinc (powdered)	20.0g

Phenolphthalein, KOH, and 100mℓ of dH_2O are refluxed, in a fume hood, with Zinc until solution is colorless (producing phenolphthalin in ~4 hours). Store stock solution refrigerated in dark bottle to which ~5g mossy zinc has been added to keep the solution in its reduced form. Remove for working solution as needed.

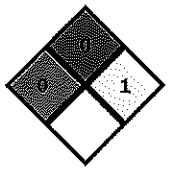
Working solution: Mix 2mℓ stock solution with 8mℓ Ethanol

Caution: Zinc is flammable. The unreacted portions and used filter paper are to be disposed of properly.

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



Form 102-BI



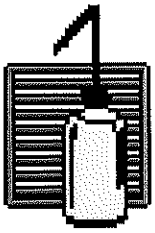
HYDROGEN PEROXIDE 3% (v/v)

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

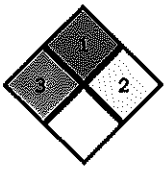
Hydrogen Peroxide (30%) 10mℓ/90mℓ nanopure dH_2O

Mix the H_2O_2 with 90mℓ of nanopure dH_2O and store at ~4°C.

Date	Initials	30% H_2O_2 Source/Lot#	Lab Lot# Reagent Name
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP
			HP



Form 103-BI

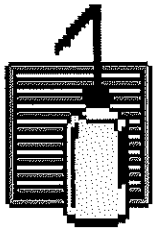


O-TOLIDINE REAGENT

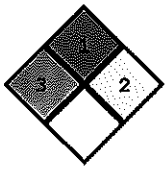
O-Tolidine	0.6g
Glacial Acetic Acid	100mℓ
Ethanol	100mℓ

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

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



Form 104-BI

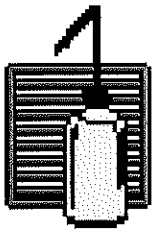


AMMONIUM HYDROXIDE (~3%)

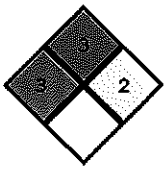
Ammonium Hydroxide (Concentrated ~30%) 10mℓ/100mℓ

Add the NH_4OH to 90mℓ of nanopure dH_2O, mix well and store at RT.

Date	Initials	NH_4OH Source/Lot#	Lab Lot# Reagent Name
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH
			AH



Form 108-BI

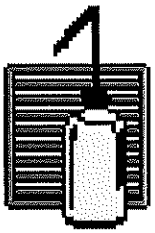


OUCHTERLONY DESTAIN

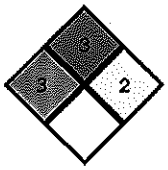
Methanol	45mℓ
Distilled water	45mℓ
Glacial Acetic Acid	10mℓ

Mix well and store refrigerated.

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



Form 110-BI

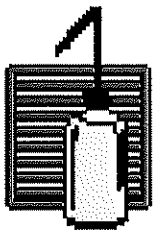


OUCHTERLONY STAIN

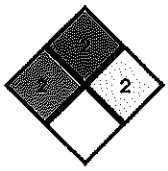
Ouchterlony Destain (Form 108-BI) 50mℓ
Coomassie Blue (Brilliant Blue R-250) 0.1g

Mix well (overnight), filter, and store at RT.

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



Form 114-BI

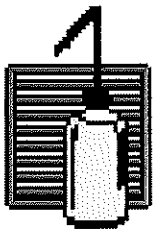


10X BRENTAMINE (SODIUM ACETATE) BUFFER

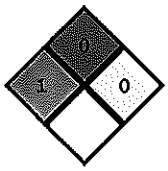
Sodium Acetate (Anhydrous)	1.2g
Acetic Acid(to adjust to pH 5)	≈400µℓ

Dissolve Sodium Acetate in 10mℓ of nanopure dH_2O. Add Acetic Acid to pH 5. Store refrigerated.

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



Form 116-BI

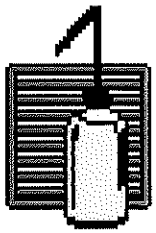


BRENTAMINE SOLUTION A

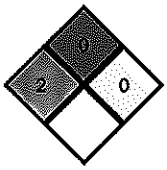
O-Dianisidine Tetrazotized (Fast Blue B Salt) 50 mg
10X buffer pH 5 5 mℓ

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

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



Form 118-BI

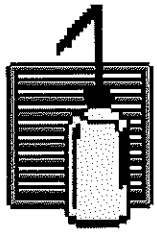


BRENTAMINE SOLUTION B

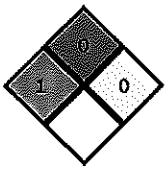
α-Naphthyl Phosphate (Disodium Salt) 50 mg

Dissolve in 5 mℓ of nanopure dH_2O. Store Refrigerated.

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



Form 120-BI

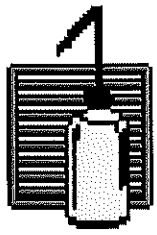


SALINE (0.85% NaCℓ)

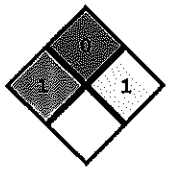
NaCℓ 4.25g/500mℓ

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

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



Form 124-BI



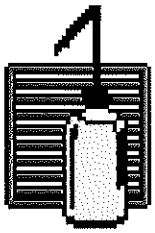
1X PHOSPHATE BUFFERED SALINE (PBS)

PBS 1 commercial pre-made packet

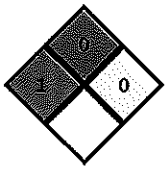
Dissolve one packet of powdered PBS in 1ℓ of nanopure dH_2O. Check that pH≅7.4, autoclave and store at RT.

If pre-made packets are not available, PBS may be prepared by dissolving 0.2g KCl, 8.0g NaCl, 0.2g KH_2PO_4, and 2.2g $Na_2HPO_4 \cdot 7H_2O$ (or 1.1g Na_2HPO_4 anhydrous) in 800mℓ nanopure dH_2O. Adjust pH to 7.4 if necessary. Q.S. to 1ℓ with nanopure dH_2O, autoclave and store at RT.

Date	Init.	1X PBS Src./Lot#	KCl Src./Lot#	NaCl Src./Lot#	KH_2PO_4 Src./Lot#	$Na_2HPO_4 \cdot 7H_2O$ Src./Lot#	Lab Lot# Reagent Name
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS
							PBS



Form 126-BI



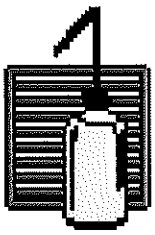
XMAS TREE STAIN SOLUTION A
(Kernechtrot Solution)

Aluminum Sulfate	5g
Nuclear Fast Red	0.1g

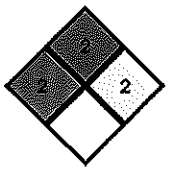
For 100mℓ, Dissolve the Aluminum Sulfate in 100mℓ **HOT** nanopure dH_2O. Immediately add the Nuclear Fast Red, mix, cool and filter (paper or ≥45µm). May be stored at RT.

May also be commercially purchased.

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



Form 128-BI



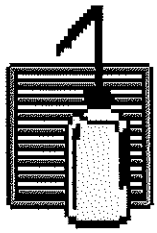
XMAS TREE STAIN SOLUTION B
(Picroindigocarmine Solution)

Saturated Picric Acid Solution	100mℓ
Indigo Carmine	0.33g

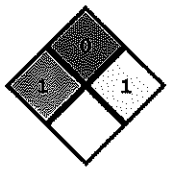
For 100mℓ, dissolve the Indigo Carmine in 100mℓ of the Picric Acid. Mix and filter (paper or ≥45µm). May be stored at RT.

May also be commercially purchased.

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



Form 132-BI

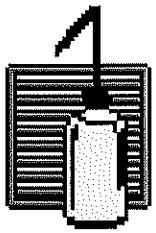


AMYLASE DIFFUSION/PHOSPHATE BUFFER (pH6.9)

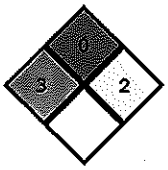
NaH_2PO_4, anhydrous	2.7g
Na_2HPO_4, anhydrous	3.9g
NaCℓ	0.2g

Mix the above with 500mℓ dH_2O, adjust pH to 6.9, and store at RT.

Date	Initials	NaH_2PO_4 Source/Lot#	Na_2HPO_4 Source/Lot#	NaCℓ Source/Lot#	Lab Lot# Reagent Name
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB
					ADB



Form 134-BI

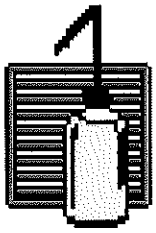


AMYLASE IODINE REAGENT

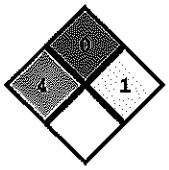
Potassium Iodide (KI)	1.65g
Iodine (I_2)	2.54g

Dissolve the above in 30mℓ nanopure dH_2O heated to ~65°C. Mix well, filter and store at 4°C in an amber bottle. Dilute 1:100 for Amylase Diffusion Test.

Date	Initials	KI Source/Lot#	I_2 Source/Lot#	Lab Lot# Reagent Name
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR
				AIR



Form 138-BI

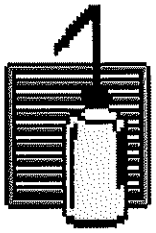


MERCURIC CHLORIDE 10% (w/v)

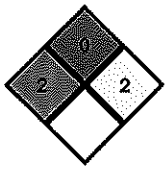
Mercuric Chloride 10g/100mℓ 95% EtOH

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

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



Form 140-BI

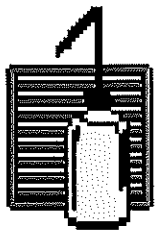


ZINC CHLORIDE 10% (w/v)

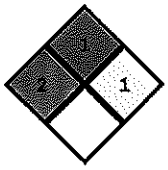
Zinc Chloride 10g/100mℓ 95% EtOH

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

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



Form 201-BI

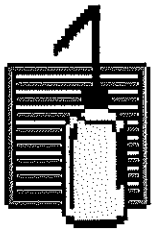


1M TRIS-HCℓ Buffer pH7.5

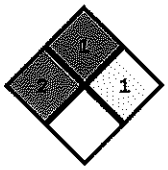
Tris Base(tris[Hydroxymethyl]amino methane) 121.1 g

Dissolve Tris in ~800 mℓ nanopure dH_2O. Adjust to pH7.5 at RT by adding concentrated HCℓ (approximately 65mℓ). Q.S. to 1ℓ with nanopure dH_2O, autoclave and store at RT.

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



Form 203-BI

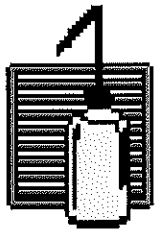


1M TRIS-HCℓ Buffer pH8

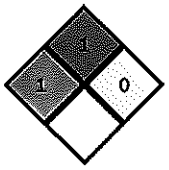
Tris Base(tris[Hydroxymethyl]amino methane) 121.1 g

Dissolve Tris in ~800 mℓ nanopure dH_2O. Adjust to pH8 at RT by adding concentrated HCℓ (approximately 45mℓ). Q.S. to 1ℓ with nanopure dH_2O, autoclave and store at RT.

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



Form 205-BI



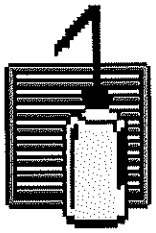
ETHYLENEDIAMINE TETRAACETIC ACID (EDTA) 0.5M

$Na_2EDTA \cdot 2H_2O$ 186.1g/ℓ

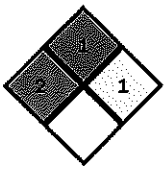
Slowly add EDTA to 800mℓ nanopure H_2O while stirring vigorously. Add ~20g of NaOH pellets to bring the pH to near 8.0. When fully dissolved adjust pH to 8.0 and bring final volume to 1ℓ. Autoclave and store at RT.

Note: EDTA will not go into solution without the pH adjustment.

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



Form 207-BI



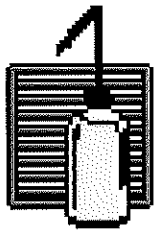
STAIN EXTRACTION BUFFER pH8
(10mM EDTA, 10mM Tris-HCℓ, 50mM NaCl, 2% SDS)

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

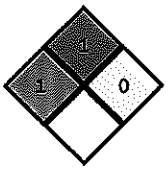
Mix the Tris-HCℓ, EDTA, NaCℓ and SDS with ~380mℓ nanopure dH_2O. Store at RT.

Note: Reagent contains SDS, do not autoclave.

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



Form 211-BI



PROTEINASE K (20mg/mℓ)

Commercial Purchase of 20mg/mℓ Solution dispensed and stored as indicated below.

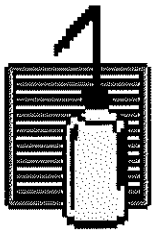
or

Proteinase K 0.2g

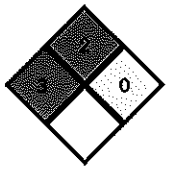
Dissolve the ProK in 10mℓ sterile nanopure dH_2O.

Dispense ~500µℓ (commercial purchase or in-house prep.) each into sterile microfuge tubes and store at ≤20°C.

Date	Initials	ProK Source/Lot#	Lab Lot# Reagent Name
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK
			PK



Form 222-BI

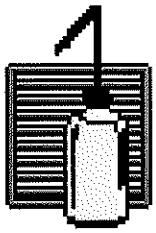


1M Sodium Acetate, pH5.2

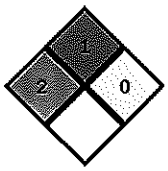
$CH_3COONa \cdot 3H_2O$ 13.6g

Dissolve the $CH_3COONa \cdot 3H_2O$ in 80mℓ nanopure dH_2O. Adjust to pH5.2 by adding glacial acetic acid (approximately 2 mℓ). Q.S. to 100mℓ with nanopure dH_2O, autoclave and store at RT.

Date	Initials	$CH_3COONa \cdot 3H_2O$ Source/Lot #	Acetic Acid Source/Lot #	Lab Lot# Reagent Name
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA
				SA



Form 223-BI



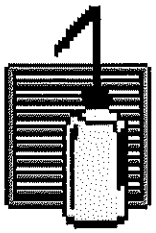
DTT SOLUTION

Dithiothreitol (DTT) 0.77g

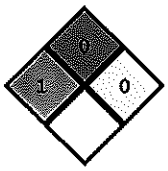
Dissolve the DTT in 5mℓ nanopure dH_2O. Add 50µℓ 1M Sodium Acetate, pH5.2. Dispense ~500µℓ each into sterile microcentrifuge tubes and store at ≅20°C.

Note: Do not autoclave.

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



Form 225-BI

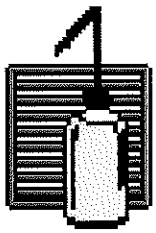


CHELEX REAGENT 5%

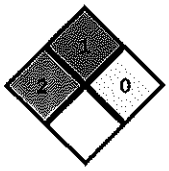
Chelex 0.5g/10mℓ

Dissolve the Chelex in 10mℓ sterile nanopure dH_2O. This solution should be freshly prepared prior to use and the remaining solution discarded after ≤3 days in refrigeator.

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



Form 229-BI

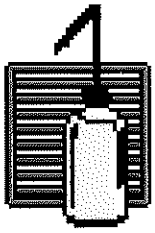


PCR-TE (TE^{-4}) BUFFER
(10mM Tris-HCl, 0.1mM EDTA)

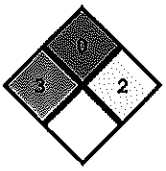
1M Tris-HCl, pH8	10mℓ
0.5M EDTA, pH8	0.2mℓ

Mix Tris-HCl and EDTA with 990mℓ nanopure dH_2O. Autoclave and store at RT.

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



Form 231-BI



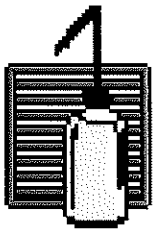
NaOH 5N

NaOH 50g

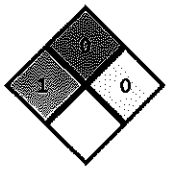
Slowly dissolve the Sodium Hydroxide in 250mℓ sterile nanopure dH_2O. Allow to cool and store at RT.

Caution: NaOH is highly caustic. This reaction generates heat.

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



Form 233-BI



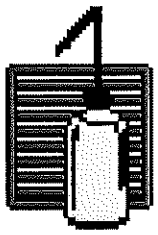
SODIUM CHLORIDE (NaCℓ) 5M

NaCℓ 146.1g/500mℓ

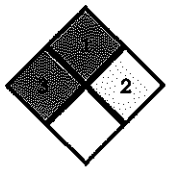
Dissolve the NaCℓ in 500 mℓ nanopure water. Sterilize by autoclaving.

May also be purchased as 5M solution.

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



Form 240-BI

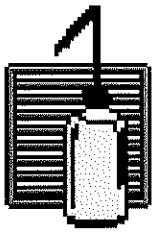


QUANTIBLOT PRE-WETTING SOLUTION (QPW)
(0.4N NaOH, 25mM EDTA)

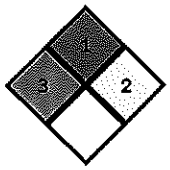
5N NaOH	40mℓ
0.5M EDTA, pH=8.0	25mℓ

Combine NaOH, EDTA and 435mℓ of nanopure dH_2O. Mix thoroughly and store at RT.

Date	Initials	NaOH Source/Lot#	0.5M EDTA Source/Lot#	Lab Lot# Reagent Name
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW
				QPW



Form 241-BI



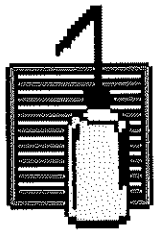
QUANTIBLOT SPOTTING SOLUTION (QSS)
(0.4N NaOH, 25mM EDTA, 0.00008% Bromothymol Blue)

5N NaOH	20mℓ
0.5M EDTA, pH=8.0	12.5mℓ
0.04% Bromothymol Blue	0.5mℓ

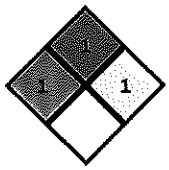
Combine NaOH, EDTA, Bromothymol Blue and 217mℓ of nanopure dH_2O. Mix thoroughly and store at RT.

Note: The solution is stable at RT for at least 3 months.

Date	Initials	NaOH Source/Lot	0.5M EDTA Source/Lot#	Bromo. Blue Source/Lot#	Lab Lot# Reagent
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS
					QSS



Form 243-BI



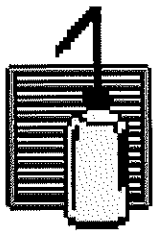
HYBRIDIZATION SOLUTION
(5X SSPE; 0.5% SDS)

10% SDS	50mℓ
20X SSPE	250mℓ

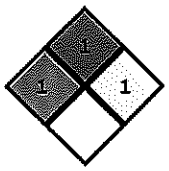
Mix the SDS and SSPE with 700mℓ nanopure dH_2O and store at RT.

Note: Hybridization Solution solids must be in solution prior to use. Warming may be required.

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



Form 245-BI



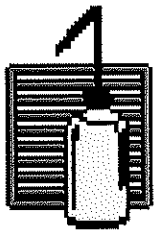
QUANTIBLOT WASH SOLUTION (QWS)
(1.5XSSPE, 0.5% SDS)

20X SSPE	150mℓ
10% SDS	100mℓ

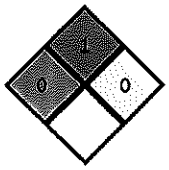
Mix the SSPE and SDS with 1750mℓ of nanopure dH_2O and store at RT.

Note: 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



Form 247-BI



CITRATE BUFFER pH5, 0.1M (QCB)

200mℓ of 10X Citrate Buffer (commercial purchase)

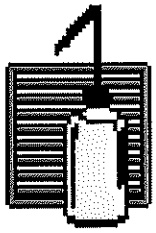
Mix 200mℓ of 10X Citrate Buffer (pH5) with 1800mℓ of nanopure dH_2O. Mix thoroughly. Check, and if necessary adjust, pH. Store at RT.

or

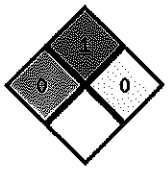
Trisodium Citrate Dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	36.8g
Citric Acid Monohydrate ($C_6H_8O_7 \cdot H_2O$)	(~12g)

Dissolve 36.8g Trisodium Citrate Dihydrate in ~1600mℓ nanopure dH_2O. Adjust to pH5.0 (±0.2) by addition of Citric Acid Monohydrate (~12g). Adjust the final volume to 2ℓ with nanopure dH_2O 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



Form 249-BI

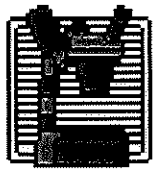


BOVINE SERUM ALBUMIN (BSA) 4%

BSA	0.4 g
PCR-TE	10 mℓ

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

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



Form 101-BI

Scientist:_____

Case Number:_____

Date:_____

BIOLOGY SCREENING SUMMARY

SAMPLE ID	BLOOD		SEMEN			SALIVA	URINE	FECES
	Chemical	Hematrace	P30	AP	MICROSCOPIC EXAM			

Form 200-BI

DNA EXTRACTION WORKSHEET

Scientist _____ **Case#** _____

Blood/Saliva Extraction

Date _____

	Lot #
1a. 500µℓ SEB	SEB _____
1b. 15µℓ Pro K	ProK _____
2. 200µℓ Chelex	Che _____
3a. 150µℓ FTA	FTA _____
3b. 150µℓ TE	TE _____

Items

Hair Extraction

Date _____

1a. 500µℓ SEB	SEB _____
1b. 20µℓ DTT	DTT _____
1c. 15µℓ Pro K	ProK _____

Fresh Bone Extraction

Date _____

1a. 500µℓ SEB	SEB _____
1b. 15µℓ Pro K	ProK _____

Old Bone Extraction

Date _____

1a. 3mℓ SEB	SEB _____
1b. 100µℓ Pro K	ProK _____

Centricon Concentration

Date _____

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

Form 202-BI

DIFFERENTIAL DNA EXTRACTION WORKSHEET

Scientist _____ **Case#** _____

Differential Extraction (EC) **Date** _____

	Lot #	Items
1a. 500µℓ SEB	SEB _____	
1b. 15µℓ Pro K	ProK _____	

Differential Extraction (SP) **Date** _____

1a.1000µℓ PBS	PBS _____	
1b. 500µℓ SEB	SEB _____	
1c. 20µℓ DTT	DTT _____	
1d. 15µℓ Pro K	ProK _____	

Centricon Concentration **Date** _____

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

Form 204-BI

QUANTIBLOT WORKSHEET

Scientist ____________ ImageID ____________

Date: ____________

Quantiblot Kit Lot# ____________ Spotting Solution Lot# QSS ____________

Membrane Source ____________ Lot# ____________

Hybridization Solution Lot# QHB ____________ 30%H_2O_2 Lot# ____________

Wash Buffer Lot# QWS ____________ Citrate Buffer Lot# QCB ____________

Chemiluminescence Reagents Lot# ____________

Record sample ID 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

Form 208-BI

DNA CONCENTRATION WORKSHEET

Scientist _____ **ImageID** _____

Date: _____

Quantification of sample DNA generated by imaging of a slot-blot should be used to bring samples to a uniform concentration where possible (0.1-0.4ng/µℓ). The following table is to be used to record: 1) the initial sample concentration (determined by dividing the ng detected on slot-blot by the volume applied), 2) the volume of sample to be diluted, 3) the amount of PCR-TE (or dH_2O) to be added to the sample and 4) the final sample concentration after dilution and 5) the amount to be amplified.

SampleID (volume)	ng Detected	µℓ Slotted	ng/µℓ Initial	µℓ Sample for Dilution	µℓ TE to be added	ng/µℓ Final	µℓ to be Amplified

Form 210-BI

STR AMPLIFICATION SET-UP

Date:_____ Scientist:_____ STR Kit Type:_____

STR Kit Lot:_____ Taq Lot:_____

Reagent	µℓ/sample	X	Master Mix #Samples	=	µℓ in Master
Rxn Buffer	_____ µℓ		_____		_____
Primers	_____ µℓ		_____		_____
H_2O	_____ µℓ		_____		_____
Taq Gold	_____ µℓ		_____		_____
Master Mix/Sample	_____ µℓ				
DNA Template	_____ µℓ				
Total Rxn Volume	_____ µℓ				

PCR TE Lot#_____

Case(s)/Samples

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

Front

Form 212-BI

STR BLIND CONTROL GENOTYPE CHECK

Blind Control Number:_____

Date:_____

LOCUS	ALLELES	LOCUS	ALLELES
D3S1358	√	THO1	√
D21S11	√	D18S51	√
Penta E	√	D5S818	√
D13S317	√	D7S820	√
D16S539	√	CSF1PO	√
Penta D	√	Amelogenin	√
vWA	√	D8S1179	√
TPOX	√	FGA	√

Correct Genotype ☒

Reviewer's Initials_____

Comments:

Form 214-BI

STR Technical Review Checklist

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 control(s), negative control(s), and sample(s).
- ☐ 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: __________

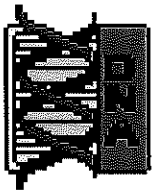
Is the following paperwork included in the CODIS Data file?

- ☐ Extraction Worksheet
- ☐ Amplification Worksheet
- ☐ Injection List
- ☐ 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 control(s), negative control(s), and sample(s).
- ☐ Genotyper plot results and table results are in agreement.
- ☐ Data certified for upload to CODIS.

Comments:



Form 310-BI

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? ☐

Form 402-QC

FORENSIC BIOLOGY EQUIPMENT MAINTENANCE/REPAIR RECORD

FILL IN ALL AVAILABLE INFORMATION.

Equipment Name/Description: _____

_____

_____

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

_____

_____

Scheduled Maintenance/Repair (circle one)

If 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:

Form 404-QC

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

Observed temperatures should fall between ±5°C of the target temperature (4°C for refrigerators and –20°C for freezers). The temperature control should be adjusted to correct for minor variations; however, If the temperature is not corrected or if it falls significantly outside the target range, it should be taken out of service and maintenance/repair performed as needed. Note: frost-free freezers (combos) will have a greater temperature range (±10°C) due to the heating and cooling cycles. Evidence will not be stored in these freezers.

DATE/INITIALS					
NANOPURE SYSTEM					
°C WATER BATH B (QB SHAKER)	**set/observed**				
°C WATER BATH C (QB REAGENTS)	**set/observed**				
°C OVEN	**set/observed**				
°C HEATING BLOCK A	**set/observed**				
°C HEATING BLOCK B	**set/observed**				

Observed temperatures should fall between ±2°C of the temperature set point. The temperature control should be adjusted to correct for minor variations; however, if the temperature is not corrected or if it falls significantly outside the target range, it should be taken out of service and maintenance/repair performed as needed.

The observed water purity for the Nanopure system should be a minimum of 18.0 mega-ohms. If the purity falls below this point, the cartridges should be changed and the system sanitized as necessary.

FORENSIC BIOLOGY MONTHLY QC

Form 406-QC

DATE/INITIALS												
AUTOCLAVE												
CLEAN												
STERILIZATION (+)												
STERILIZATION (-)												
LABORATORY AND OTHER EQUIPMENT												
EYE WASH STATION												
ARTEL PCS2												
CLEAN CENTRIFUGES												
CLEAN PIPETS												
LAB CLEANED												

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

Note: See the Artel User's Manual for calibration instructions and pass/fail criteria. If the instrument fails the calibration check it should be serviced.

Autoclave sterilization is checked by the observation of microbial growth in the (+) control (non-sterilized) and a lack of growth in the (-) control (sterilized) samples. See the BTSure product insert for test instructions and growth indicators. If sterilization is not achieved, the autoclave should be serviced.

Form 408A-QC

FORENSIC BIOLOGY QUARTERLY QC

I. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 9700

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

See User's Manual for test procedures.

Temperature Non-Uniformity

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

Calibration Verification

SETPOINT VALUE			
A6	85°C	45°C	PASS / FAIL

II. VERIFICATION TESTS FOR GENEAMP PCR SYSTEM 480

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

See User's Manual for test procedures.

Temperature Uniformity (File 32)

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

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

95°C Readings

High - Low: (____) - (____) = ____ Acceptable value is < 1°C.
Average ____ Acceptable value is within ± 1°C of target temperature.

40°C Readings

High - Low: (____) - (____) = ____ Acceptable value is < 1°C.
Average ____ Acceptable value is within ± 1°C of target temperature.

Temperature Calibration Verification (File 33)

Observed °C at 95 (____) - 0.10 = (____) [Block Average]
Acceptable Range is 94.1-95.9°C

Observed °C at 40) (____) +0.01°C = (____) [Block Average]
Acceptable Range is 39.1 - 40.9°C

Diagnostic Files Quality Control Record
Test #1: Display Check ____
Test #4: Chiller Test _____ (0.85 - 1.90°/sec)
Prior to test #5 call up File #35 and run for 10 minutes.
Test #5: Sensor Test _____ (< 0.5°C)
Test #6: Overshoot _____ (<0.5°C)
Test #7: Undershoot _____ (<2.0°C)

PLACE DOCUMENT IN PCR QC BINDER

Form 408B-QC

FORENSIC BIOLOGY QUARTERLY QC

SCIENTIST: ___________ **QC DATE:** _____________

IV. Chemical Safety Shower Check _______

V. Forensic Biology Reagent Balance Check

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

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

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

Form 410-QC

QC ABACARD® HEMATRACE® KIT

HEMATRACE® KIT LOT:_____ **DATE RECEIVED:**_____

SCIENTIST:_____ **QC DATE:**_____

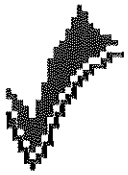
Perform test as usual with one $2mm^2$ cutting and one 2mm thread from known bloodstain. Record results (include time it took for positive rxn to be visible). If available, attach photo documentation and place in Forensic Biology QC binder.

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

The $2mm^2$ cutting sample must have a positive reaction within 10 minutes for passing. The 2mm thread should ideally be positive within 10 minutes but is used primarily as a sensitivity indicator of the given test lot. The kit may still be deemed as passing without a positive result for the thread.

QA/QC PASSED: YES ☐ **NO** ☐

Comments:



Form 412-QC

QC OneStep ABACARD® p30 KIT

ABACARD® p30 KIT LOT:_____ **DATE RECEIVED:**_____

SCIENTIST:_____ **QC DATE:**_____

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

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

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

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

QA/QC PASSED: YES ☐ **NO**☐

Comments:

Form 420-QC

QC STR KITS

STR KIT:_____ DATE RECEIVED:_____

KIT MANUFACTURER:_____ KIT LOT #:_____

LAB LOT#:_____SCIENTIST:_____QA/QC DATE:_____

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

Perform extraction of one Blind Control and amplify as usual with reagent blank and controls. Genescan® and Genotyper® data will be analyzed as usual and the quality of results reflected in the comments section as appropriate and necessary. A pass will be achieved by obtaining the expected results for each of the samples run and data of acceptable quality (e.g. sufficient RFUs).

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.

Form 422-QC

INSTRUMENT: MENDEL ROS

310 INJECTION LOG

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

Form 426-QC

ANNUAL NIST QC RUN

SCIENTIST: ________________ **QC DATE:** ____________

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

The Genescan® and Genotyper® Data will be analyzed as usual and quality of results will be reflected in the comments or 'passed' areas as appropriate and necessary. Passing results are obtained by achieving the expected results for the given NIST sample(s). 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: