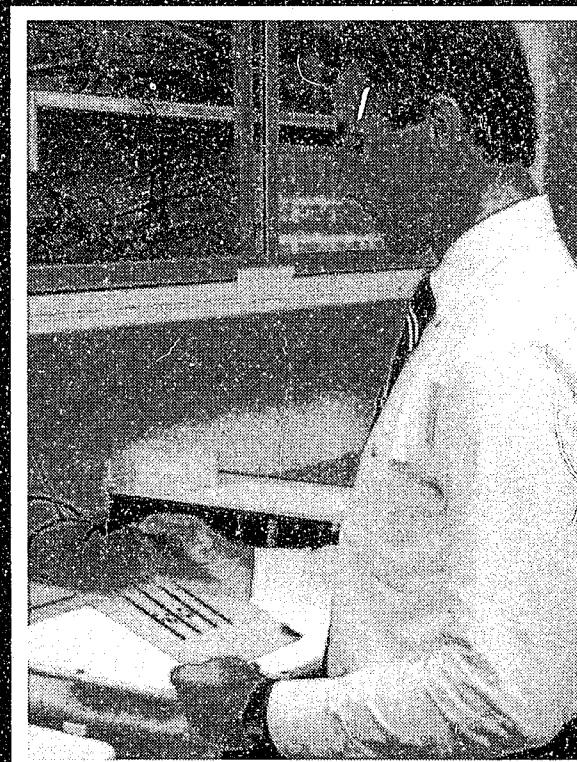
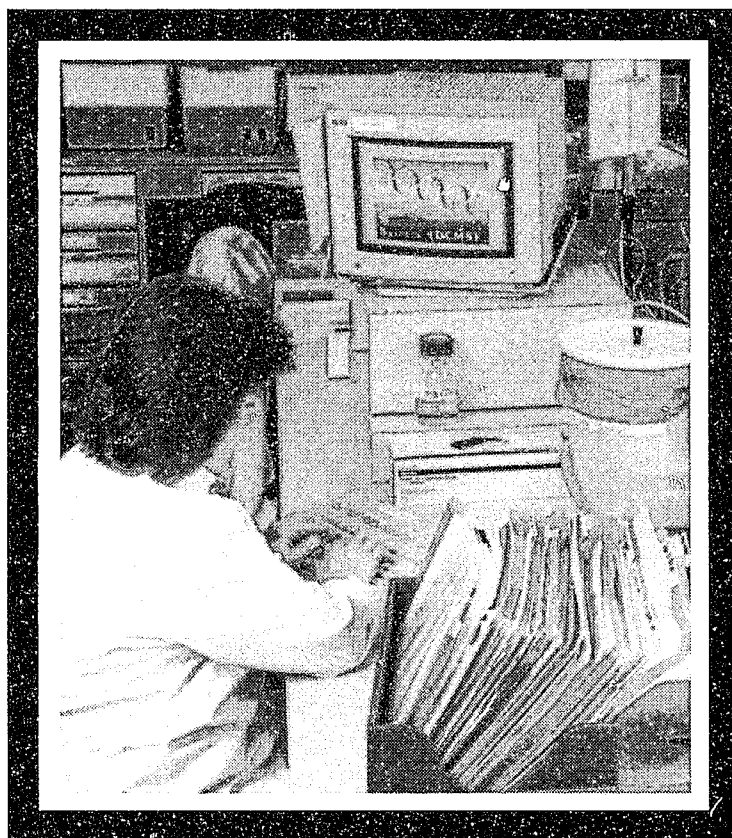




CRIME LABORATORY DIGEST

APRIL 1995

Volume 22, Number 2



Guidelines for a
Quality Assurance Program
for DNA Analysis

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Focus on Quality Assurance

Message from the Assistant Director in Charge of the FBI Laboratory

As I settle into my position as head of the FBI Laboratory, I am learning to appreciate the extremely dynamic nature of forensic science. It is very satisfying to work in a profession in which an international community is constantly developing new and improved methods to apply forensic science in support of the criminal justice system. In addition, fundamental forensic practices are evolving to further increase professional integrity. I am referring to quality assurance programs and the countless benefits to be gained by implementing such programs in the forensic sciences.

I am impressed by the growing commitment of laboratory directors and laboratory personnel to comply with quality assurance programs that are emerging throughout laboratories worldwide. The FBI Laboratory is also working diligently to implement a number of organizational policy changes to insure quality in all of our work. Although the FBI Laboratory's commitment to providing superior forensic examination services is renowned, more standardized, formal guidelines for analytical procedures are now being developed to guarantee quality assurance.

In August 1994, I established the Quality Assurance Unit in the Forensic Science Research and Training Center at the FBI Academy in Quantico, Virginia. This move represents a permanent resolve by the FBI Laboratory to provide quality service as we move into the 21st century. The Quality Assurance Unit will help to promote and monitor proper laboratory practices that apply primarily to forensic examination techniques. Through the implementation of a comprehensive quality assurance program, the Quality Assurance Unit will be able to insure that uniform quality assurance guidelines are followed when performing forensic examinations. Through the use of proficiency tests and annual audits, the Quality Assurance Unit will be in a position to monitor overall laboratory activities and recommend procedures that define, standardize, and improve laboratory practices. Through the use of reliable and sound laboratory practices and programs, the FBI Laboratory will be able to meet the challenges of future laboratory accreditations or personnel certifications.

The Quality Assurance Unit will also manage the occupational safety and health program for the FBI Laboratory. Insuring compliance with the Occupational Safety and Health Administration and Environmental Protection Agency standards will be a major responsibility. In addition, protecting

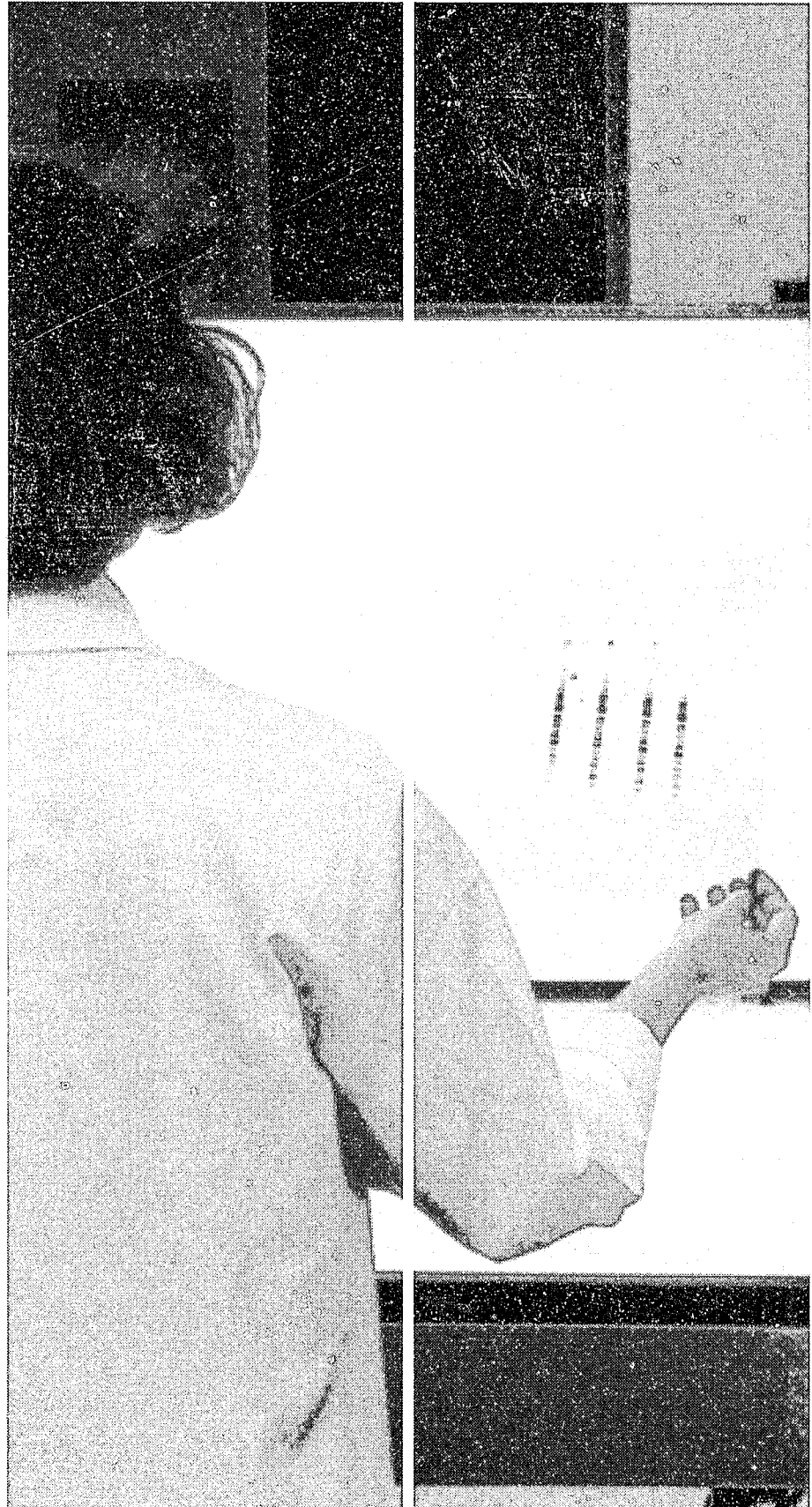
employees from potential hazards while working in the laboratory or at crime scenes will be an integral part of the program. Continued liaison of the Quality Assurance Unit with the FBI Laboratory's Evidence Response Team Unit and Evidence Response Teams in both the Laboratory and in FBI field offices will help provide services which reflect state-of-the-art safety and evidence collection practices. Increased safety training for Evidence Response Teams and other federal, state, and local law enforcement agencies will also be a major focus of the Quality Assurance Unit.

"As the Quality Assurance Unit develops comprehensive quality assurance and safety programs, it will hopefully become a resource for the forensic community for information on laboratory safety and health issues, crime scene safety and health issues, environmental hazards, proficiency testing, and overall management of quality assurance programs."

Once the Quality Assurance Unit was established, the first initiative was to staff the unit as quickly as possible with skilled professionals, and this effort is ongoing. As the Quality Assurance Unit develops comprehensive quality assurance and safety programs, it will hopefully become a resource for the forensic community for information on laboratory safety and health issues, crime scene safety and health issues, environmental hazards, proficiency testing, and overall management of quality assurance programs.

While the successful implementation of quality assurance programs requires significant dedication of human and financial resources as well as administrative support, the benefits are obvious. These benefits are even more apparent when we consider the consequences of not implementing quality assurance programs. In the end, quality assurance simply allows us to have greater confidence in our work and allows us to focus on the exciting aspect of our profession — fact finding through the analysis of forensic evidence.

Milton E. Ahlerich



Guidelines for a Quality Assurance Program for DNA Analysis

Prepared by

Technical Working Group on DNA Analysis Methods

Bruce Budowle, Group Chair
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Quantico, VA

Introduction to 1995 Revised Guidelines

As noted in the introduction to the 1991 edition of the *Guidelines for a Quality Assurance Program for DNA Analysis*, published in the April 1991 issue of the *Crime Laboratory Digest* (Vol. 18, No. 2, pp. 44-75), it was recognized that changes in the quality assurance standards for DNA testing would be necessary to accommodate evolving technology and laboratory practices.

Since the publication of the 1991 guidelines, a number of proposed changes to the guidelines have been submitted to the Technical Working Group on DNA Analysis Methods (TWGDAM) Quality Assurance (QA) Subcommittee. As a result of evolving laboratory experience and practices, as well as the advent of mitochondrial DNA analysis technology, it was determined that a review of the current guidelines was necessary.

During the January 1995 meeting of TWGDAM, a number of proposed changes to the guidelines were evaluated by the QA Subcommittee. The proposed changes were submitted in writing and were accompanied by a justification for each change. Based on the evaluation of the proposed changes and the supporting justification, the recommendations of the QA Subcommittee were forwarded to the entire TWGDAM committee for discussion. Following the discussion, each proposed change was voted upon by the TWGDAM members.

A two-thirds majority was required for the adoption of each proposed change. As a result of this voting, revisions to the following sections of the 1991 guidelines were adopted: 4.1.3, 4.1.5.10, 4.4.2.1, 5.3.2, 7.2.2, 7.3, 7.5.1.3, 7.5.1.4 (deleted), and 10.1. The 1995 revised edition of the *Guidelines for a Quality Assurance Program for DNA Analysis* follows.

1. Planning and Organization

1.1 Goals: It is the goal of the laboratory's program to:

1.1.1 Provide the users of laboratory services access to DNA typing of selected biological materials associated with official investigations using DNA testing.

1.1.2 Ensure the quality, integrity, and reliability of the DNA typing data and its presentation through the implementation of a detailed quality assurance (QA) program.

1.2 Objectives: It is the objective of the QA program to ensure that:

1.2.1 The analytical testing procedures and reporting of DNA typing are monitored by means of quality control (QC) standards, proficiency tests, and audits on a routine basis.

1.2.2 The entire DNA typing procedure is operating within the established performance criteria and that the quality and validity of the analytical data are maintained.

1.2.3 Problems are noted and corrective action is taken and documented.

1.3 Authority and Accountability

1.3.1 Organization Structure – Defines the relationships within the laboratory between individuals, job responsibilities, and operational units. It defines the relationship of the QA program to DNA analysis and related laboratory operations as well as to the laboratory management.

1.3.2 Functional Responsibilities – The job function and responsibility for each position within the laboratory should be clearly established. It should specify and describe the lines of responsibility for developing, implementing, recording, and updating the QA program.

1.3.3 Levels of Authority – Clear lines of authority and accountability should be established between personnel responsible for the QA program and those assigned to manage and perform the DNA analysis. It should be established as to who may take what action, whether approval is required, and from whom approvals are needed.

2. Personnel

2.1 Job Descriptions

The job descriptions for all DNA personnel should include responsibilities, duties, and skills.

2.2 Qualifications

The education, training, experience, and qualifying criteria of technical personnel within the DNA testing laboratory will be formally established by each laboratory. Supervisors or technical leaders and examiner/analysts must demonstrate the ability to critically evaluate and interpret the evidence, results, and data. The minimum requirements for those individuals are specified as follows.

2.2.1 Qualifying Procedure

It is highly desirable that these individuals undergo a formal qualifying procedure which reviews and documents that prerequisite criteria have been satisfied prior to the assumption of duties. These criteria should include:

- 2.2.1.1 Knowledge of the scientific principles, techniques, and literature of DNA typing as demonstrated by course work and/or written or oral examination.
 - 2.2.1.2 Practical laboratory skills in the performance of DNA analysis as demonstrated by observation and successful analytical results.
 - 2.2.1.3 Competency of individuals engaged in DNA analysis as demonstrated by the successful completion of proficiency testing.
 - 2.2.1.4 Competency of supervisors/technical leaders as demonstrated by the successful completion of proficiency testing — designed to evaluate interpretational skills.
- 2.2.2 Maintaining Qualification – There must be a procedure for the periodic review of continuing education, proficiency testing, and performance of personnel.

2.2.3 Supervisor/Technical Leader

If the supervisor alone does not meet the following criteria, the laboratory must have a technical leader or employ a consultant who satisfies all the criteria or who, in combination with the qualifications of the supervisor, satisfies the criteria. The supervisor/technical leader or other designated qualified individual must regularly review the laboratory work product and must be available for consultation. It is highly desirable that at least one individual possess all of these qualifications.

- 2.2.3.1 Education – Must have a minimum of a BA/BS or its equivalent in a biological, chemical, or forensic science and have received credit for courses in genetics, biochemistry, and molecular biology (molecular genetics or recombinant DNA technology) or other subjects which provide a basic understanding of the foundation of forensic DNA analysis.
- 2.2.3.2 Training – Must have, at a minimum:
 - (a) Training in the fundamentals of forensic biology.
 - (b) Documented training in DNA analysis with individuals, agencies, or other laboratories in a program that includes the methods, procedures, equipment, and materials used in forensic DNA analysis and their applications and limitations (ASCLD 1985).
- 2.2.3.3 Experience – Must have a minimum of 2 years of experience as a forensic biology examiner/analyst and meet all the requirements in Section 2.2.4.3.
- 2.2.3.4 Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Attendance at seminars, courses, or professional meetings is highly desirable. Laboratory management must provide the opportunity to comply with these requirements.

2.2.4 Examiner/Analyst

- 2.2.4.1 Education – Must have a minimum of a BA/BS degree or its equivalent in a biological, chemical, or forensic science and have received credit for courses in genetics, biochemistry, and molecular biology (molecular genetics, recombinant DNA technology) or other subjects which provide a basic understanding of the foundation of forensic DNA analysis.
- 2.2.4.2 Training – Must have, at a minimum:
 - (a) Training in the fundamentals of forensic biology.
 - (b) Training in DNA analysis with individuals, agencies, or other laboratories in a program that includes the methods, procedures, equipment, and materials used in forensic DNA analysis and their applications and limitations (ASCLD 1985).
- 2.2.4.3 Experience – Must include, at a minimum:
 - (a) One year of forensic biology experience.
 - (b) Prior to independent case work analysis using DNA technology, the examiner/analyst must have adequate forensic DNA laboratory experience, including the successful analysis of a range of samples typically encountered in forensic case work. This typically requires 6 months of experience in a DNA laboratory.
- 2.2.4.4 Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Attendance at seminars, courses, or professional meetings is highly desirable. Laboratory managers must provide the opportunity to comply with these requirements.

2.2.5 Technicians

- 2.2.5.1 Technicians involved in performing analytical techniques related to DNA analysis should have a minimum of a BS/BA degree (or equivalent) and receive on-the-job training by a qualified analyst. Technicians will not interpret DNA typing results, prepare final reports, or provide testimony concerning such.
- 2.2.5.2 Technicians not performing analytical techniques should have the experience and education commensurate with the job description.

3. Documentation

The DNA laboratory must maintain documentation on all significant aspects of the DNA analysis procedure, as well as any related documents or laboratory records that are pertinent to the analysis or interpretation of results, so as to create a traceable audit trail. This documentation will serve as an archive for retrospective scientific inspection, reevaluation of the data, and reconstruction of the DNA procedure. Documentation must exist for the following topic areas:

3.1 Test Methods and Procedures for DNA Typing

This document must describe in detail the protocol currently used for the analytical testing of DNA. This protocol must identify the standards and controls required, the date the procedure was adopted, and the authorization for its use. Revisions must be clearly documented and appropriately authorized.

3.2 Population Data Base – To include number, source, and ethnic and/or racial classification of samples.

- 3.3 Quality Control of Critical Reagents (such as commercial supplies and kits which have expiration dates) – To include lot and batch numbers, manufacturer's specifications, and internal evaluations.
- 3.4 Case Files/Case Notes – Must provide foundation for results and conclusions contained in formal report.
- 3.5 Data Analysis and Reporting
- 3.6 Evidence Handling Protocols
- 3.7 Equipment Calibration and Maintenance Logs
- 3.8 Proficiency Testing
- 3.9 Personnel Training and Qualification Records
- 3.10 Method Validation Records
- 3.11 Quality Assurance and Audit Records
- 3.12 Quality Assurance Manual
- 3.13 Equipment Inventory
- 3.14 Safety Manuals
- 3.15 Material Safety Data Sheets
- 3.16 Historical or Archival Records
- 3.17 Licenses and Certificates

. Validation

4.1 General Considerations for Developmental Validation of the DNA Analysis Procedure

- 4.1.1 Validation is the process used by the scientific community to acquire the necessary information to assess the ability of a procedure to reliably obtain a desired result, determine the conditions under which such results can be obtained, and determine the limitations of the procedure. The validation process identifies the critical aspects of a procedure which must be carefully controlled and monitored.
- 4.1.2 Validation studies must have been conducted by the DNA laboratory or scientific community prior to the adoption of a procedure by the DNA laboratory.
- 4.1.3 Once an RFLP procedure has been validated, appropriate studies of limited scope (*e.g.*, population studies, human DNA control value determination) must be available for each new locus used. A similar standard should be maintained when adding new loci to the different PCR-based techniques (*e.g.*, addition of short tandem repeat (STR) locus to a validated STR procedure).
- 4.1.4 The DNA primers, probe(s), or oligonucleotides selected for use in the forensic DNA analysis must be readily available to the scientific community.

4.1.5 The validation process should include the following studies: 1) *Report of a Symposium on the Practice of Forensic Serology* (1987) and 2) Budowle *et al.* (1988).

- 4.1.5.1 Standard Specimens – The typing procedure should have been evaluated using fresh body tissues and fluids obtained and stored in a controlled manner. DNA isolated from different tissues from the same individual should yield the same type.
- 4.1.5.2 Consistency – Using specimens obtained from donors of known type, evaluate the reproducibility of the technique both within the laboratory and among different laboratories.
- 4.1.5.3 Population Studies – Establish population distribution data in different racial and/or ethnic groups.
- 4.1.5.4 Reproducibility – Prepare dried stains using body fluids from donors of known types and analyze to ensure that the stain specimens exhibit accurate, interpretable, and reproducible DNA types or profiles that match those obtained on liquid specimens.
- 4.1.5.5 Mixed Specimen Studies – Investigate the ability of the system to detect the components of mixed specimens and define the limitations of the system.
- 4.1.5.6 Environmental Studies – Evaluate the method using known or previously characterized samples exposed to a variety of environmental conditions. The samples should be selected to represent the types of specimens to be routinely analyzed by the method. They should resemble actual evidence materials as closely as possible so that the effects of factors such as matrix, age, and degradative environment (temperature, humidity, UV) on a sample are considered.
- 4.1.5.7 Matrix Studies – Examine prepared body fluids mixed with a variety of commonly encountered substances (*e.g.*, dyes, soil) and deposited on commonly encountered substrates (*e.g.*, leather, denim).
- 4.1.5.8 Nonprobative Evidence – Examine DNA profiles in nonprobative evidentiary stain materials. Compare the DNA profiles obtained for the known liquid blood versus questioned blood deposited on typical crime scene evidence.
- 4.1.5.9 Nonhuman Studies – Determine if DNA typing methods designed for use with human specimens detect DNA profiles in nonhuman source stains.
- 4.1.5.10 Minimum Sample – Where appropriate, establish quantity of DNA needed to obtain a reliable typing result.
- 4.1.5.11 On-Site Evaluation – Set up newly developed typing methods in the case-working laboratory for on-site evaluation of the procedure.
- 4.1.5.12 It is essential that the results of the developmental validation studies be shared as soon as possible with the scientific community through presentations at scientific/professional meetings. It is imperative that details of these studies be available for peer review through timely publications in scientific journals.

4.2 Characterization of Loci

During the development of a DNA analysis system, basic characteristics of the loci must be determined and documented (Baird 1989; AABB Standards Committee 1990).

- 4.2.1 Inheritance - DNA loci used in forensic testing shall have been validated by family studies to demonstrate the mode of inheritance. Those DNA loci used in parentage testing should have a low frequency of mutation and/or recombination.
- 4.2.2 Gene Mapping - The chromosomal location of the polymorphic loci used for forensic testing shall be submitted to or recorded in the Yale Gene Library or the International Human Gene Mapping Workshop.
- 4.2.3 Detection - The molecular basis for detecting the polymorphic loci shall be documented in the scientific or technical literature.
 - 4.2.3.1 For RFLP, this includes the restriction enzyme and the probes used.
 - 4.2.3.2 For PCR, this includes the primers and probes, if used.
- 4.2.4 Polymorphism - The type of polymorphism detected shall be known.
- 4.3 Specific Developmental Validation of RFLP Procedures
 - 4.3.1 Restriction - The conditions and control(s) needed to ensure complete and specific restriction must be demonstrated.
 - 4.3.2 Separation - Parameters for the reproducible separation of DNA fragments must be established.
 - 4.3.3 Transfer - Parameters for the reproducible transfer of DNA fragments must be established.
 - 4.3.4 Detection - The hybridization and stringency wash conditions necessary to provide the desired degree of specificity must be determined.
 - 4.3.5 Sizing - The precision of the sizing procedure must be established.
- 4.4 Specific Developmental Validation of PCR-Based DNA Procedures
 - 4.4.1 Amplification
 - 4.4.1.1 The PCR primers must be of known sequence.
 - 4.4.1.2 Conditions and measures necessary to protect preamplification samples from contamination by post-PCR materials should be determined (See Section 7.5).
 - 4.4.1.3 The reaction conditions such as thermocycling parameters and critical reagent concentrations (primers, polymerase, and salts) needed to provide the required degree of specificity must be determined.
 - 4.4.1.4 The number(s) of cycles necessary to produce reliable results must be determined.
 - 4.4.1.5 Potential for differential amplification must be assessed and addressed.
 - 4.4.1.6 Where more than one locus is amplified in one sample mixture, the effects of such amplification on each system (alleles) must be addressed and documented.
 - 4.4.2 Detection of PCR Product

The validation process will identify the panel of positive and negative controls needed for each assay described as follows.

4.4.2.1 Characterization without Hybridization

- (a) When a PCR product is characterized directly, appropriate standards for assessing the alleles shall be established.
- (b) When a PCR product is characterized by direct sequencing, appropriate standards for assessing the sequence shall be established.

4.4.2.2 Characterization with Hybridization

- (a) Hybridization and stringency wash conditions necessary to provide the desired degree of specificity must be determined.
- (b) For assays in which the amplified target DNA is to be bound directly to a membrane, some mechanism should be employed to ensure that the DNA has been applied to the membrane.
- (c) For assays in which the probe is bound to the membrane, some mechanism should be employed to show that adequate amplified DNA is present in the sample (*e.g.*, a probe which reacts with any amplified allele or a product yield gel).

4.5 Internal Validation of Established Procedures (ASCLD 1986)

Prior to implementing a new DNA analysis procedure or an existing DNA procedure developed by another laboratory that meets the developmental criteria described under Section 4.1, the forensic laboratory must first demonstrate the reliability of the procedure in-house. This internal validation must include the following:

- 4.5.1 The method must be tested using known samples.
- 4.5.2 If a modification which materially effects the results of an analysis has been made to an analytical procedure, the modified procedure must be compared to the original using identical samples.
- 4.5.3 Precision (*e.g.*, measurement of fragment lengths) must be determined by repetitive analyses to establish criteria for matching.
- 4.5.4 The laboratory must demonstrate that its procedures do not introduce contamination which would lead to errors in typing.
- 4.5.5 The method must be tested using proficiency test samples. The proficiency test may be administered internally, externally, or collaboratively.

5. Equipment, Materials, and Facilities

5.1 Equipment

Only suitable and properly operating equipment should be employed. Where critical parameters of equipment operation are identified in the validation procedure, monitoring of those parameters should be conducted and documented in the manner necessary to maintain successful operation of the typing technique.

- 5.1.1 Inventory – A list of equipment requiring calibration and monitoring for DNA analysis, which includes the manufacturer, model, serial number, agency inventory number, and acquisition dates, should be maintained.

- 5.1.2 Operation Manual – The manufacturer's operation manual should be readily available.
- 5.1.3 Calibration, Maintenance Procedures, and Logs – There should be written calibration and maintenance procedures and schedules. There should be a permanent log of calibration and maintenance of equipment essential for DNA typing (e.g., thermal cyclers and water baths).
- 5.1.4 Dedicated Equipment – Dedicated equipment should be readily identifiable as such.

5.2 Materials and Reagents

Chemicals and reagents should be of suitable quality, correctly prepared, and demonstrated to be compatible with the methods employed.

- 5.2.1 Logs must be maintained of commercial supplies and kits which have expiration dates (e.g., amplification kits, probes, or enzymes), as indicated in Section 3.3.
- 5.2.2 Formulation - There must be a written procedure for the formulation of reagents, standards, and controls.
- 5.2.3 Labeling Requirements - Labels should include identity, concentration, date of preparation, identity of individual preparing reagents, special storage requirements, and expiration date, where appropriate.
- 5.2.4 A current inventory of supplies and materials should be maintained to include information on supplier, catalog number, lot number, date received, and storage location.
- 5.2.5 Dedicated Materials and Reagents – Dedicated materials and reagents should be readily identifiable as such.
- 5.2.6 Glassware and Plastic Supplies Preparation – There should be specific procedures for cleaning, preparation, and sterilization.

5.3 Laboratory Facilities for PCR Analysis

A PCR laboratory will require special laboratory configuration and sample handling (*AmpliType™ User Guide* 1990).

- 5.3.1 Examination Work Area – Area(s) for examination, photography, and microscopy must be separated in time or space from the extraction and amplification setup areas.
- 5.3.2 Extraction Work Area(s) – This area is for sample extraction, concentration, and digestion. It must be physically separate from the amplified DNA work area and be separated in time or space from the PCR setup area. An extraction area for samples containing low DNA levels (e.g., telogen hairs, old bone) should be separated in time or space from other DNA extraction areas.
- 5.3.3 PCR Setup Work Area – This area is isolated from the extraction area by time or in space to ensure that the reaction mix cocktails are prepared in a clean environment. This area must be physically separated from the amplified DNA work area.
- 5.3.4 Amplified DNA Work Area – This area is separated physically in the laboratory for containment of amplified DNA product. This area includes the amplification area with the thermal cycler and space for all procedures utilizing the product for typing (e.g., gel electrophoresis, hybridization, and washing). Amplified DNA should be stored and disposed of in this area. All equipment and reagents used in this area should be dedicated and should not be used in either the extraction or PCR setup areas.
- 5.3.5 Decontamination – There must be written procedures for the cleaning and decontamination of facilities and equipment from DNA and PCR product DNA.

6. Evidence Handling Procedures

Evidence and samples from evidence must be collected, received, handled, sampled and stored so as to preserve the identity, integrity, condition, and security of the item.

- 6.1 Sample Labeling – Each sample must be labeled with a unique identifier in accordance with agency policy.
- 6.2 Chain of Custody – A clear, well-documented chain of custody must be maintained from the time the evidence is first received until it is released from the laboratory (ASCLD 1986).
- 6.3 Sample Handling and Storage – Each agency will prepare a written policy to ensure that evidence samples (including isolated DNA and membranes) will be handled, processed, and preserved so as to protect against loss, contamination, and deleterious change. Disposition of evidence should be in accordance with law and agency regulations. Refer to Section 5.3 for PCR sample handling considerations.

7. Analytical Procedures

7.1 Sample Evaluation and Preparation

- 7.1.1 General characterization of the biological material should be performed prior to DNA analysis. Evidence samples submitted should be evaluated to determine the appropriateness for DNA analysis.
- 7.1.2 When semen is identified, a method of differential extraction should be employed, and, where appropriate, each of the DNA fractions typed (see Section 4.1.5.10).
- 7.1.3 Testing of evidence and evidence samples should be conducted to provide the maximum information with the least consumption of the sample. Whenever possible, a portion of the original sample should be retained or returned to the submitting agency, as established by laboratory policy.

7.2 DNA Isolation

- 7.2.1 The DNA isolation procedure should protect against sample contamination.
- 7.2.2 The effectiveness of the DNA isolation procedure should be evaluated by periodic use of an appropriate source of human DNA.

7.3 Procedures for Estimating DNA Recovery:

Where appropriate, a procedure should be used for estimating the quality (extent of DNA degradation) and quantity of DNA recovered from the specimens. One or more of the following procedures may be employed to evaluate the effectiveness of the DNA recovery.

- 7.3.1 Yield Gel – Yield gels must include a set of high molecular weight DNA calibration standards for quantitative estimate of yield.
- 7.3.2 UV Absorbance – Absorbance and wavelength standards or a high molecular weight DNA calibration standard may be used.

7.3.3 Fluorescence – Approximate quantification of extracted DNA can be accomplished by comparison with known concentrations of high molecular weight DNA.

7.3.4 Hybridization – Quantitation with human/primate specific probes requires an appropriate set of human DNA standards.

7.4 Analytical Procedures for RFLP Analysis

7.4.1 Restriction Enzymes

7.4.1.1 Prior to its initial use, each lot of restriction enzyme should be tested against an appropriate viral, human, or other DNA standard which produces an expected DNA fragment pattern under standard digestion conditions. The restriction enzyme should also be tested under conditions that will reveal contaminating nuclease activity.

7.4.1.2 Demonstration of Restriction Enzyme Digestion – Digestion of extracted DNA by the restriction enzyme should be demonstrated using a test gel which includes:

- (a) Size Marker – Determines approximate size range of digested DNA.
- (b) Human DNA Control – Measures the effectiveness of restriction enzyme digestion of genomic human DNA.

7.4.2 Analytical Gel – The analytical gel used to separate restriction fragments must include the following:

7.4.2.1 Visual Marker – Visual or fluorescent markers which are used to determine the end point of electrophoresis.

7.4.2.2 Molecular Weight Size Markers – Markers which span the RFLP size range and are used to determine the size of unknown restriction fragments. Case samples must be bracketed by molecular weight size marker lanes.

7.4.2.3 Human DNA Control – A documented positive human DNA control of known type which produces a known fragment pattern with each probe and serves as a systems check for the following functions:

- (a) Electrophoresis quality and resolution
- (b) Sizing process
- (c) Probe identity
- (d) Hybridization efficiency
- (e) Stripping efficiency

7.4.2.4 A procedure should be available to interpret altered migration of DNA fragments.

7.4.3 Southern Blots/Hybridization – The efficiency of blotting, hybridizations, and stringency washes are monitored by the human DNA control and size markers.

7.4.4 Autoradiography – The exposure intensity is monitored by the use of multiple X-ray films or by successive exposures in order to obtain films of the proper intensity for image analysis.

7.4.5 Image and Data Processing – The functioning of image and data processing is monitored by the human DNA control allelic values.

7.5 Analytical Procedures for PCR-Based Techniques

7.5.1 Internal Controls and Standards

The laboratory's QC guidelines should contain specific protocols to assess critical parameters in normal operations which include the following:

7.5.1.1 Negative controls to be included with each sample set are:

- (a) A reagent blank
- (b) An amplification blank

7.5.1.2 A human DNA known type must be introduced at the amplification step as a positive control and carried through the remainder of the typing.

7.5.1.3 Where appropriate, controls should be collected from the evidence and should be processed in the same manner as evidence samples.

7.5.1.4 To characterize amplified fragment length polymorphisms, markers which span the allele size range must be used. Case samples must be bracketed by marker lanes.

8. Case Work Documentation, Interpretation, Report Writing, and Review

Laboratories should have policies, checks, and balances in place which ensure the reliability and completeness of the documentation, data analysis, reports, and review process.

8.1 Case Work Documentation

Documentation must be in a form such that a competent analyst or supervisor/technical leader, in the absence of the primary analyst, would be able to evaluate what was done and to interpret the data.

Documentation must include, but is not limited to, data obtained through the analytical process. It should also include information regarding the packaging of the evidence upon receipt and the condition of the evidence itself, paying particular attention to those factors which are relevant to the preservation of the biological material. All documentation of procedures, standards, and controls used, observations made, results of the tests performed, charts, graphs, photographs, autoradiographs, communications, etc., which are used to support the analyst's conclusions must be preserved as a record according to written laboratory policy. Results should be preserved by photography, autoradiography, or other suitable means.

8.2 Interpretation of Data

Laboratories should have general guidelines for interpretation of data for each method of DNA analysis.

8.2.1 Evaluation of Controls

8.2.1.1 Guidelines for interpreting and acting upon positive and/or negative control results.

8.2.1.2 Guidelines for statistical monitoring of the human DNA control if appropriate to the procedure (ANSI/ASQC A1-1987, ANSI/ASQC Z1.1-1985, ANSI/ASQC Z1.2-1985, ANSI/ASQC Z1.3-1985; AT&T Technologies 1985; Westgard *et al.* 1981; Gryna 1979; Bicking and Gryna 1979; National Bureau of Standards 1966).

8.2.2 Evaluation of Samples

- 8.2.2.1 The basis for concluding when samples are or are not the same type or when the results of the analysis are inconclusive or uninterpretable should be established.
- 8.2.2.2 For RFLP analysis, confirmation of visual matches of the restriction fragment bands must be made by quantitative analysis based on tolerance limits.
- 8.2.2.3 Statistical Evaluation – The frequency of occurrence for the DNA profile should be calculated using a scientifically valid method from an established population data base.

8.3 Report Writing

Contents – It is highly desirable that reports contain the following:

8.3.1 Case Identifier

8.3.2 Identity of Examiner/Analyst

8.3.3 Date of Report

8.3.4 The DNA Locus (defined by the Nomenclature Committee of the International Gene Workshop), as identified by particular probe(s) or sequence(s)

8.3.5 Restriction Enzyme, Primer Pair, or Other Descriptor of the Methodology

8.3.6 Results

8.3.7 Conclusions

8.3.8 Statistical Evaluation

8.3.9 Signature of the Reporting Analyst

8.4 Review

Data, documentation, and reports must be reviewed independently by a second qualified individual. Prior to issuing a report, both individuals must agree on the interpretation of the data and the conclusions derived from that data.

9. Proficiency Testing

Proficiency testing is used periodically to demonstrate the quality performance of the DNA laboratory and serves as a mechanism for critical self-evaluation. This will be accomplished by the analysis and reporting of results from appropriate biological specimens, submitted to the laboratory as open and/or blind case evidence.

All specimens submitted as part of an open or blind proficiency test must be analyzed and interpreted according to the DNA analysis protocol approved by the laboratory for use at the time of the proficiency test.

Participation in a proficiency testing program is a critical element of a successful QA program and is an essential requirement for any laboratory performing forensic DNA analysis. A forensic laboratory involved in DNA analysis may establish its own proficiency testing program or establish a program in cooperation with another forensic laboratory.

The DNA laboratory should participate in proficiency testing programs, conducted by outside institutions or provided by other reputable sources, which are appropriately designed for forensic DNA analysis.

9.1 Open Proficiency Testing

Open proficiency test specimens are presented to the laboratory and its staff as proficiency specimens and are used to demonstrate the reliability of the laboratory's analytical methods as well as the interpretive capability of the examiner/analyst. Participation in an open proficiency test program is the primary means by which the quality performance of the DNA laboratory is judged and is an essential requirement if a DNA laboratory is to perform case work.

9.1.1 Personnel

Open proficiency testing pertains to those laboratory examiners/analysts and technicians actively engaged in DNA testing.

9.1.2 Frequency

Open proficiency tests must be submitted to the DNA testing laboratory such that each examiner/analyst, as well as those technicians involved in performing analytical techniques related to DNA analysis, are tested at least twice a year.

9.1.3 Specimens

Each open proficiency test may consist of dried specimens of blood and/or other physiological fluids, either singly or as a mixture. Each sample to be tested should contain an amount sufficient so that a conclusion can be drawn from the results of the analysis.

For those DNA procedures which use electrophoretic analysis for identification of the DNA polymorphisms, the number of specimens included in the proficiency test should be such that all may be accommodated on a single analytical gel.

For those DNA analysis procedures which use PCR for DNA amplification, coupled with a nonelectrophoretic method for the identification of the DNA polymorphism, an equivalent number of samples should be tested.

Those samples which comprise proficiency tests intended for PCR-based techniques must include the appropriate negative controls as specified in Section 7.5.1.3.

9.1.4 Sample Preparation, Storage, and Distribution

- (a) All specimens and proficiency tests should be uniformly prepared using materials and methods that ensure their integrity and identity.
- (b) All open proficiency test specimens will be prepared on washed cotton cloth, cotton swabs, or other suitable material.
- (c) Each specimen and set must be labeled with a unique identifier that should be independently verified by at least one other person to ensure proper assignment of the identifier.
- (d) A portion of each specimen used to prepare the open proficiency test should be retained by the preparing laboratory for possible referee analysis and comparison if circumstances dictate.
- (e) A person in the DNA laboratory, as designated by laboratory manager, should acknowledge the receipt of each proficiency test and assign it to the DNA laboratory staff.

9.2 Blind Proficiency Testing

Ideally, blind proficiency test specimens should be presented to the testing laboratory through a second agency. These samples should appear to the examiner/analyst as routine evidence. The blind proficiency test serves to evaluate all aspects of the laboratory examination procedure, including evidence handling, examination/testing, and reporting. It is highly desirable that the DNA laboratory participate in a blind proficiency test program, and every effort should be made to implement such a program.

9.2.1 Personnel

Blind proficiency testing pertains only to personnel previously qualified by their laboratory to conduct DNA testing.

9.2.2 Frequency

Those laboratories which have implemented a blind testing program and are engaged in the analysis and interpretation of DNA profiles should be tested by a blind proficiency test at least once a year.

9.2.3 Specimens

Each blind proficiency test will consist of liquid or dried specimens of blood and/or other physiological fluids, either singly or as a mixture. Each sample to be tested should contain an amount sufficient so that a conclusion can be drawn from the results of the analysis.

For those DNA procedures which use electrophoretic analysis for identification of the DNA polymorphisms, the number of specimens included in the proficiency test should be such that all may be accommodated on a single analytical gel.

For those DNA analysis procedures which use PCR for DNA amplification, coupled with a nonelectrophoretic method for the identification of the DNA polymorphism, an equivalent number of samples should be tested.

Those samples which comprise proficiency tests intended for PCR-based techniques must include the appropriate negative controls as specified in Section 7.5.1.3.

9.2.4 Sample Preparation, Storage, and Distribution

- (a) All specimens and proficiency tests should be uniformly prepared using materials and methods that ensure their integrity and identity.
- (b) All blind proficiency tests should be prepared so as to realistically simulate the characteristics of actual case work.
- (c) The identity of each specimen and set must be independently verified by at least one other person to ensure proper assignment of the identifier.
- (d) A portion of each specimen used to prepare the blind proficiency test should be retained by the preparing laboratory for possible referee analysis and comparison if circumstances dictate.
- (e) Once prepared, all samples must be packaged separately, and sets must be stored until submission to the testing agency so as to maintain their integrity and condition.

- (f) The QA coordinator, or other individual designated by the laboratory, will make all necessary arrangements for the covert submission of the blind proficiency test, including supporting documentation and agency contact.
- (g) Unless specifically authorized by the laboratory director or QA coordinator, prior to the analysis and reporting of the blind proficiency results, no person in the laboratory undergoing blind proficiency testing should be aware of the ongoing blind proficiency test or the personnel involved.

9.3 Documentation of Proficiency Test Results

9.3.1 Open Proficiency Tests

At a minimum, the following proficiency test data and information should be collected and submitted to the QA coordinator or other designated individual for evaluation:

- (a) Open proficiency test set identifier
- (b) Identity of examiner/analyst
- (c) Dates of analysis and completion
- (d) Copies of all data sheets and notes
- (e) Photographs of yield, post-restriction (digestion) test, and analytical gels and/or dot blots as appropriate
- (f) Lot numbers of primers or probes and the sequence of use
- (g) Lot numbers of commercially prepared supplies or kits
- (h) Original or duplicate autorads, where appropriate
- (i) Computer imaging sizing data, where appropriate
- (j) Likelihood estimates for samples
- (k) Results/conclusions

9.3.2 Blind Proficiency Tests

The report of the DNA laboratory will be sent to the submitting agency in the normal course of laboratory operations, and prior arrangements should be made for its immediate forwarding to the QA coordinator or other designated individual.

Upon receipt of the forwarded DNA report, the QA coordinator or other designated individual will require that the DNA laboratory provide the data and documentation specified in Section 9.3.1. In addition, documentation on the receipt, storage, handling, and chain of custody may also be requested for review. The blind proficiency test evidence may also be recovered from the testing or submitting agency and examined for proper documentation and handling. If the testing laboratory retains portions of the tested materials or products of its analysis, these should be examined for proper documentation and storage.

9.4 Review and Reporting of Proficiency Test Results

The QA coordinator or other designated individual will review all test materials and compare results to the information from the manufacturer of the test. The QA coordinator will provide a written summary report for each proficiency test to the examining examiner/analyst and other appropriate individuals as established by the laboratory policy. This review should be conducted in a timely manner. All original notes, records, and other data pertaining to the open proficiency test results should be retained according to laboratory policy.

9.5 Corrective Action

The specific policies, procedures, and criteria for any corrective action taken as a result of a discrepancy in a proficiency test should be clearly defined and approved by the appropriate individuals in accordance with established laboratory policies.

9.5.1 Authority and Accountability

It is the responsibility of the QA coordinator or designated individual to assure that discrepancies are acknowledged and that any corrective action is documented.

In the event of an unresolved disagreement between the designated QA individual and DNA laboratory, the matter should be referred to the laboratory director.

9.5.2 Administrative Error

Any significant discrepancy in a proficiency test determined to be the result of administrative error (e.g., clerical error, sample confusion, improper storage, inaccurate documentation, etc.) will be corrected according to established laboratory policy.

9.5.3 Systematic Error

Any significant discrepancy in a proficiency test determined to be the result of a systematic error (e.g., equipment, materials, environment) may require a review of all relevant case work since the DNA unit's or laboratory's last successfully completed proficiency test. Once the cause of the discrepancy has been identified and corrective action has been taken, all examiners/analysts should be made aware of the appropriate corrective action in order to minimize the recurrence of the discrepancy.

9.5.4 Analytical/Interpretative Error

- (a) Any significant discrepancy in a blind or open proficiency test result determined to be the consequence of an analytical/interpretative discrepancy should prohibit the individual(s) involved in producing the discrepant result from further examination of case evidence until the cause of the problem is identified and corrected. The QA coordinator or designated individual will determine the need to audit prior cases, according to established laboratory policy.
- (b) Before resuming analysis or interpretation of case work, an additional set of open proficiency samples must be successfully completed by the individual responsible for the discrepancy.

9.6 Documentation

The results of all proficiency tests will be maintained by the DNA laboratory according to established laboratory policy.

10. Audits

Audits are an important aspect of the QA program. They are an independent review conducted to compare the various aspects of the DNA laboratory's performance with a standard for that performance (Mills 1989; Sayle 1988). The audits are not punitive in nature but are intended to provide management with an evaluation of the laboratory's performance in meeting its quality policies and objectives.

- 10.1 Audits or inspections should be conducted at least once every 2 years by individuals separate from and independent of the DNA testing laboratory. It is highly desirable that at least one auditor be from an outside agency.
- 10.2 Records of each inspection should be maintained and should include the date of the inspection, the area inspected, the name of the person conducting the inspection, findings and problems, remedial actions taken to resolve existing problems, and the schedule of next inspection.

11. Safety

- 11.1 Policy – The DNA testing laboratory shall operate in strict accordance with the regulations of the pertinent federal, state, and local health and safety authorities.
- 11.2 Written Manuals – Written general laboratory safety and radiation safety manuals shall be prepared by the laboratory and be made available to each member of the DNA analysis laboratory and/or other persons affected (*Code of Federal Regulations* 1988a, 1988b; Bond 1987; Gibbs and Kasprisin 1987; Sax and Lewis 1987; National Fire Protection Association 1986; National Research Council 1981; Wang *et al.* 1975; Steere 1971).
- 11.3 Material Safety Data Sheets (MSDS) – There should be a file of MSDS received from the manufacturer for all chemicals used in the laboratory. These data sheets should be readily available to all laboratory personnel.
- 11.4 Storage and Disposal – All chemicals, supplies, and radioactive materials must be stored, used, and disposed of under conditions recommended by the manufacturer and in a manner conforming to established safety requirements.

Glossary

Allele: In classical genetics, one of the alternate forms of the gene at a particular locus. In DNA analysis, the term "alleles" is commonly extended to include DNA fragments of variable length and/or sequence which may have no known transcriptional product but are detected in a polymorphic system.

Amplification: Increasing the number of copies of a desired DNA sequence.

Amplification Blank: A control that consists of only amplification reagents without the addition of sample DNA. This control is used to detect DNA contamination of the amplification reagents and materials.

Anneal: The formation of double strands from two complementary single strands of DNA and/or RNA. In the second step of each PCR cycle, primers bind or anneal to the 3' ends of the target sequence.

Autograph: An image produced on a piece of film by radioactive or chemiluminescent material.

Cycle: The PCR cycle consists of three steps: 1) denaturation of the template, 2) annealing of primers to complementary sequences at an empirically determined temperature, and 3) extension of the bound primers by a DNA polymerase.

Denaturation: The conversion of helical, double strands of DNA to single strands by heat or chemical reagents. Denaturation by heat is the first step of each PCR cycle.

Differential Extraction: A step-wise extraction procedure designed to separate intact sperm heads from lysed sperm and other cell types. The separation generally results in an enrichment of sperm DNA in one cell fraction relative to the other cell fraction. The separate fractions can be analyzed individually.

DNA Contamination: The unintentional introduction of exogenous DNA into a DNA sample or PCR reaction prior to amplification.

Extension: The covalent linkage of deoxyribonucleoside triphosphates in a template-directed manner by DNA polymerase. Linkage is in a 5' to 3' direction starting from the 3' end of bound primers. PCR primers are extended one nucleotide at a time by a DNA polymerase during each PCR cycle.

Genome: The genetic constituent of an organism, contained in the chromosome.

Hybridization: The process of complementary base pairing between two single strands of DNA and/or RNA.

Kilobase (kb): Unit of 1,000 base pairs of DNA or 1,000 bases of RNA.

Locus: The site on a chromosome where a gene or a defined sequence is located.

Polymerase Chain Reaction (PCR): An enzymatic process by which a specific region of DNA is replicated during repetitive cycles (see cycle).

Polymorphism: A variation in the sequence at a given locus where no one allele exists in more than 99 percent of the population.

Primers: Small oligonucleotides complementary to the 3' ends of the target sequence. A pair of primers specifies the boundaries of the region being amplified during the PCR.

Probe: A fragment or sequence of DNA that hybridizes to a complementary sequence of nucleotides in another single-strand nucleic acid (target).

Quality Assurance: Those planned or systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.

Quality Audit: A systematic and independent examination and evaluation to determine whether quality activities and results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives.

Quality Control: The day-to-day operational techniques and the activities used to fulfill requirements of quality.

Quality Plan: A document setting out the specific quality practices, resources, and activities relevant to a particular product, process, service, contract, or project.

Reagent Blank Control: This control consists of all reagents used in the test process minus any sample. This is used to detect DNA contamination of the analytical reagents and materials.

Restriction Enzyme: A bacterial enzyme that recognizes a specific palindromic sequence of nucleotides in double-stranded DNA and cleaves both strands; also called a restriction endonuclease.

Restriction Fragment Length Polymorphism (RFLP): The variation occurring in the length of DNA fragments generated by a specific restriction enzyme.

Southern Blot: DNA that has been separated by electrophoresis, transferred from the gel to an immobile support (e.g., nitrocellulose or nylon), and bonded onto the support in single-strand form for hybridization.

Sterile Technique: In the context of PCR work, it does not include flaming of bottles and pipets. Gloves, sterile supplies, and clean work areas are required in addition to the use of separate pipet tips for each reagent addition to each reaction tube. Additional explanation of the sterile technique for PCR work can be found in the *Amplitype™ User Guide* (1990, Section 2 – Laboratory Setup).

Stringency: The conditions of hybridization that increase the specificity of binding between two single-strand portions of nucleic acids, usually the probe and the immobilized fragment. Increasing the temperature or decreasing the ionic strength results in increased stringency.

Substrate Control: Unstained material adjacent to, or representative of, the area upon which the biological material is deposited.

Variable Number of Tandem Repeats (VNTR): Copies of a DNA sequence arranged in succession in a chromosome.

References

- AABB Standards Committee. P7.000 DNA polymorphism testing. In: *Standards for Parentage Testing Laboratories*. 1st ed. American Association of Blood Banks, Arlington, VA, 1990.
- Alwan, L. C. and Bissell, M. G. Time series modeling for quality control in clinical chemistry, *Clinical Chemistry* (1988) 34:1396-1406.
- American National Standard ANSI/ASQC A1-1987. *Definitions, Symbols, Formulas, and Tables for Control Charts*. American Society for Quality Control, Milwaukee, WI, 1987.
- American National Standard ANSI/ASQC Q90-1987. *Quality Management and Quality Assurance Standards – Guidelines for Selection and Use*. American Society for Quality Control, Milwaukee, WI, 1987 a).
- American National Standard ANSI/ASQC Q90-1987. *Quality Management and Quality System Elements – Guidelines*. American Society for Quality Control, Milwaukee, WI, 1987(b).
- American National Standard ANSI/ASQC Z1.1-1985. *Guide for Quality Control Charts*. American Society for Quality Control, Milwaukee, WI, 1985.
- American National Standard ANSI/ASQC Z1.2-1985. *Control Chart Method of Analyzing Data*. American Society for Quality Control, Milwaukee, WI, 1985.
- American National Standard ANSI/ASQC Z1.3-1985. *Control Chart Method of Controlling Quality During Production*. American Society for Quality Control, Milwaukee, WI, 1985.
- American National Standard ANSI/ASQC Z-1.15-1979. *Generic Guidelines for Quality Systems*. American Society for Quality Control, Milwaukee, WI, 1979.
- American National Standard ANSI/ASQC A3-1978. *Quality Systems Terminology*. American Society for Quality Control, Milwaukee, WI, 1978.
- American National Standard ASQC Standard C1-1968. *Specification of General Requirements of a Quality Program*. American Society for Quality Control, Milwaukee, WI, 1968.
- AmpliType™ User Guide (for the AmpliType™ HLA DQα Forensic DNA Amplification and Typing Kit), Section 2 – Laboratory Setup. Cetus Corporation, Emeryville, CA, 1990.
- ASCLD. *Guidelines for Forensic Laboratory Management Practices*. American Society of Crime Laboratory Directors, September 1986.
- ASCLD. *ASCLD Accreditation Manual*. American Society of Crime Laboratory Directors, Laboratory Accreditation Board, February 1985.
- AT&T Technologies. *Statistical Quality Control Handbook*. AT&T Technologies, Indianapolis, IN, May 1985.
- Baird, M. *Quality Control and American Association of Blood Bank Standards*. Presented at the American Association of Blood Banks National Conference, Leesburg, VA, April 17-19, 1989.
- Bicking, C. A. and Gryna, F. M., Jr. Process control by statistical methods. In: *Quality Control Handbook*. 3d ed., ed. J. M. Juran, Section 23. McGraw-Hill, New York, 1979.
- Bond, W. W. Safety in the forensic immunology laboratory. In: *Proceedings of the International Symposium on Forensic Immunology*, 101-109. US Government Printing Office, Washington, DC, 1987.
- Box, G. E. P. and Bissard, S. The scientific context of quality improvement, *Quality Progress* (1987) 20(6):54-61.

- Bradford, L. W. Barriers to quality achievement in crime laboratory operations, *Journal of Forensic Sciences* (1980) 25:902-907.
- Brunelle, R. L., Garner, D. D., and Wineman, P. L. A quality assurance program for the laboratory examination of arson and explosive cases, *Journal of Forensic Sciences* (1982) 27:774-782.
- Budowle, B., Deadman, H. A., Murch, R. S., and Baechtel, F. S. An introduction to the methods of DNA analysis under investigation in the FBI Laboratory, *Crime Laboratory Digest* (1988) 15:8-21.
- Bussolini, P. L., Davis, A. H., and Geoffrion, R. R. A new approach to quality for national research labs, *Quality Progress* (1988) 21(1):24-27.
- Code of Federal Regulations, Title 10, Part 19 – Notices, Instructions, and Reports to Workers; Inspections.* US Government Printing Office, Washington, DC, 1988(a).
- Code of Federal Regulations, Title 10, Part 20 – Standards for Protection Against Radiation.* US Government Printing Office, Washington, DC, 1988(b).
- Ford, D. J. Good laboratory practice, *Laboratory Practice*, (1988) 37(9):29-33.
- Gautier, M. A. and Gladney, E. S. A quality assurance program for health and environmental chemistry, *American Laboratory* (July 1987) 17-22.
- Gibbs, F. L. and Kasprisin, C. A. *Environmental Safety in the Blood Bank.* American Association of Blood Banks, Arlington, VA, 1987.
- Gryna, F. M., Jr. Basic statistical methods. In: *Quality Control Handbook*. 3d ed., ed. J. M. Juran, Section 22. McGraw-Hill, New York, 1979.
- Hay, R. J. The seed stock concept and quality control for cell lines, *Analytical Biochemistry* (1988) 171:225-237.
- Juran, J. M. Quality policies and objectives. In: *Quality Control Handbook*. 3d ed., ed. J. M. Juran, Section 3. McGraw-Hill, New York, 1979.
- Kenney, M. L. Quality assurance in changing times: proposals for reform and research in the clinical laboratory field, *Clinical Chemistry* (1987) 33:328-336.
- Kidd, G. J. What quality means to an R & D organization. In: *41st Annual Quality Congress Transactions.* American Society for Quality Control. Milwaukee, WI, May 4-6, 1987.
- Kilshaw, D. Quality assurance. 1. Philosophy and basic principles, *Medical Laboratory Science* (1986) 43:377-381.
- Kilshaw, D. Quality assurance. 2. Internal quality control, *Medical Laboratory Science* (1987a) 44:73-83.
- Kilshaw, D. Quality assurance. 3. External quality assessment, *Medical Laboratory Science* (1987b) 44:178-186.
- Mills, C. A. *The Quality Audit: A Management Evaluation Tool.* American Society for Quality Control, Milwaukee, WI, 1989.
- National Bureau of Standards. The place of control charts in experimental work. In: *Experimental Statistics.* *National Bureau of Standards Handbook 91.* US Government Printing Office, Washington, DC, 1966.
- National Fire Protection Association. *Standard on Fire Protection for Laboratories Using Chemicals.* National Fire Protection Association, Batterymarch Park, Quincy, MA, 1986.

- National Research Council. *Prudent Practices for Disposal of Chemicals from Laboratories*. National Research Council's Committee on Hazardous Substances in the Laboratory, National Academy Press, Washington, DC, 1983.
- National Research Council. *Prudent Practices for Handling Hazardous Chemicals in Laboratories*. National Research Council's Committee on Hazardous Substances in the Laboratory, National Academy Press, Washington, DC, 1981.
- Pereira, M. Quality assurance in forensic science, *Forensic Science International* (1985) 28:1-6.
- Report of a Symposium on the Practice of Forensic Serology, Method Evaluation (Topic 4)*. Sponsored by the California Department of Justice Bureau of Forensic Services, California Association of Criminalists, and the UNISYS Corporation, 1987.
- Ruzicka R. K. Documentation: Configuration management. In: *Quality Control Handbook*. 3d ed., ed. J. M. Juran, Section 19. McGraw-Hill, New York, 1979.
- Sax, N. I. and Lewis, R. J. *Hazardous Chemicals Desk Reference*. Van Nostrand Reinhold, New York, 1987.
- Sayle, A. J. *Management Audits: The Assessment of Quality Management Systems*. 2d ed. American Society for Quality Control, Milwaukee, WI, 1988.
- Simpson, J. National Bureau of Standards approach to quality, *Test and Measurement World* (December 1983) 38.
- Steere, N. V., ed. *CRC Handbook of Laboratory Safety*. 2d ed. The Chemical Rubber Company, Cleveland, OH, 1971.
- Taylor, J. K. *Quality Assurance of Chemical Measurements*. Lewis Publishers, Chelsea, MI, 1987.
- Taylor, J. K. The quest for quality assurance, *American Laboratory* (October 1985) 67-75.
- Wang, C. H., Willis, D. L., and Loveland, W. D. *Radiotracer Methodology in the Biological, Environmental, and Physical Sciences*. Prentice-Hall, Englewood Cliffs, NJ, 1975.
- Westgard, J. O., Barry, P. L., Hunt, M. R., and Groth, T. A multi-rule Shewart chart for quality control in clinical chemistry, *Clinical Chemistry* (1981) 27:493-501.
- Whitehead, T. P. and Woodford, F. P. External quality assessment of clinical laboratories in the United Kingdom, *Journal of Clinical Pathology* (1981) 34:947-957.

Notes from the Technical Working Group on DNA Analysis Methods

The Technical Working Group on DNA Analysis Methods (TWGDAM) was formed to address the development and implementation of forensic DNA analysis methods in public crime laboratories throughout North America. This group has met with considerable success in the coordination, conduct, and reporting of experimental studies supporting restriction fragment length polymorphism (RFLP) analysis. In addition, TWGDAM members have published guidelines for conducting the RFLP and polymerase chain reaction (PCR)-based tests for use by the crime laboratory community.

As new methods and techniques arise from the fields of molecular biology and population genetics, it has been considered a responsibility of TWGDAM to examine these advances for their potential to enhance existing procedures or open new routes to the genetic typing of biological evidence. During the February 1993 meeting of TWGDAM, representatives of participating laboratories were organized into several working groups. Each working group was tasked with examining emerging issues and developments pertinent to a specific area of DNA typing. Groups were designated to study the following areas: (1) additional guidelines for quality assurance (QA) and quality control (QC) of DNA analyses, (2) enhancements to RFLP analysis, (3) new approaches to using PCR, and (4) methods for the typing of mitochondrial DNA (mtDNA).

The following is a summary of the activities of the various TWGDAM working groups which resulted from the July 1994 meeting:

QA/QC WORKING GROUP

The QA/QC Working Group did not meet.

RFLP WORKING GROUP

I. Members in Attendance and Laboratory Affiliations

Harold Deadman – FBI Laboratory (Group Chair)
Eric Buel – Vermont Department of Public Safety
Joseph Caruso – Indianapolis-Marion County Forensic Services Agency
Thomas Grant – Missouri State Highway Patrol
Kenneth Konzak – California Department of Justice
Donald MacLaren – Washington State Patrol
David McClure – South Carolina Law Enforcement Division
James Pollock – Florida Department of Law Enforcement
Renee Romero – Washoe County Sheriff's Office
Clement Smetana – US Army Criminal Identification Laboratory
Christine Tomsey – Pennsylvania State Police
Gary Verret – Royal Canadian Mounted Police
Linda Watson – Maryland State Police

II. Summary of Meeting

A. Evaluation of a Temperature Stable *Hae III* Restriction Enzyme furnished by Cellmark Diagnostics, Inc. (update) – James Pollock

1. The South Carolina Law Enforcement Division observed a retardation of bands with the temperature stable *Hae III* compared with normal banding patterns with regular *Hae III*. This retardation appeared to be dependent upon the use of ethidium bromide (EtBr). If EtBr was used, no difference was observed in banding patterns with the two *Hae III* enzymes. Band retardation was observed only when EtBr was not used. A possible explanation is that some type of protein-DNA complex, which would have less mobility, is being maintained in the absence of EtBr. If EtBr was intercalated into the DNA, it might prevent the protein-DNA binding. No other work was reported on this issue.

B. Population Studies of New Probes – Thomas Grant

1. Population data on D5S110 were distributed to TWGDAM members. Five thousand individuals were probed. The data have yet to be analyzed..
2. Additional probes being considered for evaluation are D7S467 and D17S26. D7S467 is fairly sensitive, but it is not as polymorphic as most probes presently used.

C. Probe Usage Survey

1. A survey was conducted to determine the number of probes used routinely in forensic laboratories. The results are as follows:

7 probes: South Carolina Law Enforcement Division
6 probes: Federal Bureau of Investigation
Florida Department of Law Enforcement
Pennsylvania State Police
Washoe County Sheriff's Office
5 probes: California Department of Justice
Indianapolis-Marion County Forensic Services Agency
Maryland State Police
Missouri State Highway Patrol
Vermont Department of Public Safety
4 probes: Royal Canadian Mounted Police

The probes used by the laboratories surveyed were D1S7, D2S44, D4S139, D5S110, D7S467, D10S28, and D17S79.

D. Reporting of Coincidental Match Probabilities

1. A survey was conducted to determine if a minimum probability was used in reporting results of a DNA comparison. While most laboratories used a minimum probability, it varied among the different laboratories and ranged from 1 in 100 million to 1 in 10 billion. A few laboratories reported the calculated probability, while one laboratory reported the largest probability in the databases used by that laboratory.
2. A survey was conducted to determine the statistical approach used by different laboratories when a three-band profile is involved in a match. Most laboratories would report the match but would not attempt to incorporate the results into the final multilocus probability.

E. Measurement Error Study (update) – Eric Buel

1. Dried bloodstains were prepared and distributed to 10 to 12 laboratories. These stains are from individuals who have large fragments (greater than 10,000 base pairs) present in some of their profiles. Only four laboratories have returned sizing data at this time. Results from all of the laboratories are needed so that appropriate statistical analyses of the data can be conducted.

F. Chemiluminescent Detection (update) – Clement Smetana

1. The US Army Criminal Identification Laboratory is attempting to develop an overnight incubation procedure that is less time-consuming. It is presently using Gibco BRL's ACES 2.0 and Lumiphos-Plus for detection. The question of when a laboratory should switch to chemiluminescence was discussed. Most laboratories agreed that the time to switch is when all probes presently used are accessible in the chemiluminescence format and when it had been demonstrated that the probes have adequate sensitivity.

G. Statistical Approaches for Criminal Paternity – Gary Verret

1. A suggested statistical approach for use in criminal paternity cases was developed by George Carmody from Carlton University. This approach is currently used by the Royal Canadian Mounted Police.

H. Discussion of Problems/Solutions Associated with RFLP Analysis

1. It was reported that precut K562 from Promega was producing extra bands. Laboratories having this problem began purchasing uncut K562 and cutting it in the laboratory.
2. Many laboratories have successfully extracted DNA from samples without using dithiothreitol (DTT). Extraction buffer without DTT seems to provide better recovery, especially with degraded samples.
3. Synthetic oligo probes (*i.e.*, D2S44 and D17S79 from Lifecodes) are generally more sensitive than purified insert counterparts. An exception has been noted with probe D2S44 and a very small allele. The synthetic oligo probe produced a one-band profile, whereas a purified insert D2S44 probe produced a two-band profile. The missing band was small (approximately 770 base pairs). For very small fragments, the flanking DNA that is present in the purified insert probe but absent in the synthetic oligo probe may be necessary to generate sufficient binding for detection. It was mentioned that Lifecodes currently cannot sell D2S44.
4. A paper recently published in *Nucleic Acids Research* claims that a single-strand cutting enzyme is involved in apoptosis (programmed cell death). Degraded DNA could contain many single-strand nicks which could affect their flexibility and perhaps their mobility. This could be why degraded DNA has slightly greater mobility than higher quality DNA.
5. Several laboratories reported DNA bands detected by ladder probes but not human probes.
6. Extra bands (usually weak) have been observed by a number of laboratories with probes D2S44 and D10S28. These bands are observed in known blood samples and seem to follow the primary bands around. They have been called "buddy" or "bloody" bands because their positions seem to be affected by the positions of the primary bands. These extra bands may result from nuclease activity clipping off a small portion of each allele while the DNA is organized in the nucleosomes in liquid blood. If this were happening, the flanking DNA that surrounds the variable number of tandem repeats (VNTR) could be responsible for organizing the DNA around the nucleosome so that hypersensitive sites open to nuclease cutting would be in the same or similar positions for each chromosome.
7. Some smearing of the size marker ladders was observed when Gibco BRL agarose was used. Other lots of the same type of agarose were fine.

PCR WORKING GROUP

I. Members in Attendance and Laboratory Affiliations

Bruce Budowle – FBI Laboratory (Group Chair)
F. Samuel Baechtel – FBI Laboratory
Jeffrey Ban – Virginia Division of Forensic Sciences
Charles Barna – Michigan State Police
Elizabeth Benzinger – Illinois State Police
David Bing – CBR Laboratories, Inc.
Catherine Comey – FBI Laboratory
George Duncan – Broward County Sheriff's Department
Marcia Eisenberg – Roche Biomedical Laboratories
Pamela Fish – Chicago Police Department
George Herrin – Georgia Bureau of Investigation
Roger Kahn – Metro-Dade Police Department
Robert Keister – Orange County Sheriff's-Coroner Department
Terry Laber – Minnesota Bureau of Criminal Apprehension
Jenifer Lindsey – FBI Laboratory
Susan Narveson – Arizona Department of Public Safety
Mark Nelson – North Carolina Bureau of Investigation
Pamela Newall – Centre of Forensic Sciences
Lawrence Presley – FBI Laboratory
Dennis Reeder – National Institute of Standards and Technology
Rebecca Reynolds – Roche Molecular Systems
Georgia Sue Rogers – Alabama Department of Forensic Sciences
Cecilia von Beroldingen – Oregon State Police
Stacy Warnecke – Kentucky State Police

II. Summary of Meeting

A. Substrate Controls – Jenifer Lindsey

1. In a validation study for envelopes, the FBI Laboratory detected faint typing dots in controls (sterile swabs) for both DQ α and Polymarker systems using an organic extraction method. It did not have any problem when the controls were extracted using Chelex[®] 100. This phenomena could cause problems in interpretation. It was suggested that a threshold study be conducted to determine the amount of DNA that needs to be present to prevent the amplification of the contaminant encountered on the control swabs.
 - a. Threshold Study
 - 1) Cut clean swabs in half.
 - 2) Extract one-half of swab using an organic extraction method. Capture the DNA using a Centricon 100[®] concentrator. Q.S. the sample to 200 μ l.
 - 3) Slot blot 20 μ l of the sample (1/10 of total).
 - 4) Amplify and type the sample.
 - 5) If any swabs show typing results, add DNA to the other half of those swabs (10 ng, 5 ng, 1.25 ng, 0.625 ng, 0.300 ng).
 - 6) Report results to Bruce Budowle within 3 months.

B. Population Estimates/PCR-Based Systems – Ceiling

1. David Bing reported a case in which the court requested that he select the most frequent frequencies from databases for his calculations.
2. It was suggested that local population studies be conducted and that the population data be collected.

C. D1S80

1. High bands, 41+ should be binned.
2. p^2 should be used for homozygotes.

D. Product Royalties and Patents

1. The issue of royalties and patents and how they will affect the cost of products remains unresolved. It is hoped that negotiations with the Human Identification Trade Association and individual companies will solve the problem. TWGDAM hopes to negotiate a solution to this problem.

E. Polymarker – Rebecca Reynolds

1. Roche Molecular Systems has updated its Polymarker population data.
2. Polymarker validation studies indicate that coamplification does not compromise results at each locus. The study compared results generated by amplifying at a single locus to those obtained from the Polymarker multiplex.
3. Polymarker loci are organized according to size. As the sample degrades, the intensities from locus to locus will show differences. However, the overall balance of the dots generally will remain constant. If hybridization temperatures are too high, one may observe intensity differences at the Gc and LDLR loci. At these loci, as the temperature increases, the intensity will decrease, thus creating an imbalance.

F. Polymarker Validation/FBI – Bruce Budowle

1. The FBI validation of the Polymarker system included the following parameters:
 - a. Cross-reaction with other species
 - b. Stability, time/sunlight
 - c. Substrates
 - d. Chemical contamination
 - e. Mixtures
 - f. Sensitivity
 - g. Tissues
 - h. Hybridization temperature
 - i. Population studies
2. The cross-reaction study showed hybridization with:
 - a. Higher primates
 - b. Low-level hybridization with goat at high DNA input levels (20 to 30 ng)
3. The tissue study indicated no problems.
4. The hybridization study demonstrated a more efficient binding at 54°C. However, at 55°C (manufacturer's recommendation), there is less cross reactivity. The FBI Laboratory will continue to use 55°C.
5. Population studies demonstrate that there are more differences between major groups than within major groups.

G. New Product Development/Roche Molecular Systems – Rebecca Reynolds

1. The next generation of strips may be a bar code format. This will allow for more loci and/or systems to be put on a strip.
2. Strips are being developed for mtDNA typing based on Mark Stoneking's sequences.
3. Strips are now available for sex markers. These markers will be incorporated into other strips in the future.

MITOCHONDRIAL DNA WORKING GROUP

I. Members in Attendance and Laboratory Affiliations

Joseph DiZinno – FBI Laboratory (Group Chair)
Charles Ginther – University of California at Berkeley
Mitchell Holland – Armed Forces Institute of Pathology
Terry Melton – Pennsylvania State University (nonmember)
Mark Stoneking – Pennsylvania State University
Mark Wilson – FBI Laboratory

II. Summary of Meeting

- A. The group discussed various issues regarding the development of mtDNA technology for forensic casework.
- B. Charles Ginther is now working in George Sensabaugh's laboratory at the University of California at Berkeley, and he recently sequenced DNA from 94 individuals from Sierra Leone. He is in the process of comparing the pattern types and distributions of these sequences with a database of African American samples from New York, NY. So far, he is observing similar mtDNA sequence pattern types and distributions in both populations.
- C. Mitchell Holland is completing a mutation rate study of 100 to 150 mother/child comparisons. No data from this study were presented at the meeting. He also is optimizing primer pairs for mtDNA amplification and modifying the Armed Forces Institute of Pathology's bone extraction protocol.
- D. Terry Melton presented mtDNA data utilizing a sequence-specific oligonucleotide hybridization technique to study subpopulation heterozygosity and its effect on determining the probabilities of random matches in forensic applications. Mark Stoneking also is beginning a mtDNA study of approximately seven generations in a closed population from Tristdan da Cunha, an isolated island in the mid-Atlantic Ocean.
- E. Mark Wilson discussed the progress of the FBI Laboratory's mtDNA research effort. The FBI Laboratory is researching multiplexing of HV1 and HV2 amplification and the use of restriction endonucleases to minimize contamination. The FBI Laboratory has established preliminary protocols for hairs, bones, and teeth and has created population databases consisting of 50 African Americans, 50 Caucasians, and approximately 40 Orientals.
- F. The group considered establishing QA/QC guidelines for laboratories using mtDNA in forensic casework. The group wants to establish these guidelines, but it was decided that more information is needed before guidelines can be established. The group will reconsider establishing QA/QC guidelines at the next TWGDAM meeting.
- G. Mitchell Holland distributed copies of the proposed US Department of Defense quality assurance program for mtDNA identification of ancient remains. He agreed to distribute the QA/QC guidelines recommended by the College of American Pathologists and the American Society of Crime Laboratory Directors to all TWGDAM mtDNA members prior to the next TWGDAM meeting. The FBI Laboratory will collect and distribute other PCR QA/QC guidelines to all mtDNA members.

- H. The group provided some basic QA/QC guidelines that might be considered for the application of mtDNA typing to forensic casework. These guidelines are as follows:
1. Physically separate extraction space should be available for anticipated low-level DNA extraction procedures.
 2. Use of a laminar flow hood for extraction and amplification setup.
 3. Use of dedicated reagent/supplies for low-level DNA extraction and amplification.
 4. Any validated typing or sequencing methodology is acceptable (automated or manual sequencing, reverse dot blot, PCR oligonucleotide ligase assay, etc).
 5. Extraction and amplification blanks should be run with all PCR amplifications in casework.
 6. Extraction and amplification of questioned samples should be performed before extraction and amplification of known samples, if performed in the same area. Questioned samples also should be sequenced before known samples.
 7. Strive for typing of HV1 and HV2 with a minimum number of ambiguities.
 8. Reference samples should be typed for all laboratory personnel involved in the process.
- I. The group briefly discussed the possibility of developing a regional laboratory structure for the forensic application of mtDNA technology. This will be discussed further at the next TWGDAM meeting.

The following 19 pages are a reprint of the Executive Summary of a report by the Counterdrug Technology Assessment Center of the Office of National Drug Control Policy. The report provides the results of the technical evaluation of the BULLETPROOF® and DRUGFIRE™ ballistic imaging systems.

Please note that the original page numbers of this report have been maintained. Although the report begins on page 51 of the *Crime Laboratory Digest*, it then follows the page sequence of the original report's Executive Summary (pages i through xviii). The main text of the report and the appendices are not included in this reprint.

Normal pagination sequence for the *Crime Laboratory Digest* resumes on page 70.

Copies of the complete report are available from the Office of National Drug Control Policy, Counterdrug Technology Assessment Center, Executive Office of the President, Washington, DC 20500.

BENCHMARK EVALUATION STUDIES of the BULLETPROOF and DRUGFIRE BALLISTIC IMAGING SYSTEMS

A Technical Evaluation with
Recommendations for Action



**Executive Office of the President
Office of National Drug Control Policy
Counterdrug Technology Assessment Center**

November 1994




EXECUTIVE OFFICE OF THE PRESIDENT
OFFICE OF NATIONAL DRUG CONTROL POLICY
Washington, D.C. 20500

Recently, the Office of Management and Budget requested that my office conduct a technical performance assessment of two ballistic imaging systems, BULLETPROOF AND DRUGFIRE. The report provides the results of the assessment of the technical performance of the two ballistic imaging and examination systems and recommendations on ways to integrate the systems into a single quite versatile system. The Counterdrug Technology Assessment Center (CTAC) within my office organized and carried out the study.

For the past seventy years, forensic experts have used the comparison microscope to examine the ballistics of weapons used in violent crimes. One by one, a Firearms Examiner would compare recovered specimens against a test specimen fired from a suspect weapon. We now have an opportunity to introduce advanced imaging system technology to assist the firearms examiner. The new approach, called ballistic imaging, provides an examiner with state-of-the-art data acquisition, image matching, image manipulation, and networked communications capabilities. With a ballistic imaging system, the examiner uses data searching and image correlation algorithms to interpret the class and individual characteristics of the ammunition under examination.

Based on my thirty years experience in police work, I am firmly convinced that the deployment of regional networks of mutually compatible ballistic imaging systems would result in a dramatic increase in linking and solving more criminal cases. The recommendations from the report should be considered as Federal guidelines for the introduction of ballistic imaging technology. To ensure compatibility with the regional networks serving their area in the future, I would encourage the directors of the more than 160 laboratories around the country to consider the recommendations contained within the report.


Lee P. Brown
Director

BENCHMARK EVALUATION STUDIES of the BULLETPROOF® and DRUGFIRE™ BALLISTIC IMAGING SYSTEMS

Executive Summary

At the request of the Office of Management and Budget (OMB), the Office of National Drug Control Policy - Counterdrug Technology Assessment Center (ONDCP/CTAC) organized an independent evaluation of two computer based ballistic imaging systems named **BULLETPROOF®** (BP) and **DRUGFIRE™** (DF). These ballistic imaging systems use the powerful searching capabilities of the computer to match the images of recovered crime scene evidence against digitized images stored in a computer database.

The BP system, used to analyze bullets, has been sponsored, in part, by the Bureau of Alcohol, Tobacco, and Firearms (BATF) of the United States Treasury Department. The DF system, used to analyze cartridge cases, has been sponsored by the Federal Bureau of Investigation (FBI) of the United States Department of Justice.

The three objectives of this independent evaluation project were to: 1) perform an independent evaluation of the BP and DF systems consisting of system performance and life cycle cost analyses, 2) perform a "redundancy analysis", and 3) perform an "integration analysis". To conduct this evaluation project, CTAC assembled an independent team of experts consisting of a project leader from the Houston Advanced Research Center (HARC), a systems engineer and cost analyst, a computer and image analyst, an optics engineer, and two Firearms Examiners. The names, affiliations, and concise resumes of the experts on the Independent Evaluation Team are listed in Appendix A.

The performance of the sophisticated image acquisition, correlation algorithms, network communications, and design of the BP and DF systems was evaluated using a standard series of computer image analysis and system evaluation criteria commonly referred to as measures of effectiveness (MOEs). These system performance MOEs included: overall system accuracy, overall processing capability, system processing speed, complexity, computer requirements, database size/restrictions, interface compatibility, network compatibility, human factors, reliability, environmental limitations, facilities requirements, and expandability. These MOEs are standard performance measures that would be used to evaluate any computer based image matching system. Additionally, a Life Cycle Cost analysis was performed on each system based on a *national scale systems deployment plan* over a five year time frame. The entire set of MOEs were agreed to and approved by BATF, FBI, and OMB. Because the functionality of the two ballistic imaging systems continues to evolve, their performance measured by the MOEs should be considered as indications of the current performance a Firearms Examiner could expect from the BP and DF systems.

Firearms Examiners have traditionally classified and identified ballistic evidence on bullets and cartridge cases from *class* and *individual* characteristics. *Class* characteristics identify a family of firearms and, in some cases, distinguish different manufacturers. The bullet *class*

characteristics include the number of land and groove impressions, direction of twist, and the land impression width. Cartridge case *class* characteristics include the location of the extractor and ejector marks, the shape of the firing pin and the firing pin drag. Thus, *class* characteristics by themselves are useful in that they can reduce a large database to a more manageable level. With a computer based ballistic imaging system, the Firearms Examiner now uses sophisticated data searching and image correlation algorithms to interpret the class and individual characteristics of the ammunition under examination.

The traditional method of ballistic evidence examination as performed by a Firearms Examiner manually compares, one by one, the recovered specimens against a test specimen fired from a suspect weapon. This current procedure for determining if a recovered firearm was used to fire one of the cartridge cases or bullets in the *open case files* is extremely time consuming. The procedure requires the examiner to physically remove the evidence from a vault, mount the test evidence specimens on a microscope, and perform an optical comparison. This comparison can be as short as thirty (30) minutes or as long as twenty (20) hours (or more) depending on the difficulty of the marks and degree of documentation required. At first glance, this does not seem significant. However, after considering the number of open case files and their broad geographical distribution, it is evident that there are considerable problems with the current examination methods. The chain of custody requirements often make it impractical to routinely analyze such evidence. Currently, unsolved open case files are only consulted when the Firearms Examiner has definitive information from the investigator that two or more cases may have involved a common firearm. Obviously, this is an infrequent occurrence. By using a ballistic imaging system, the computer retains the *images* of the evidence and can transmit that *image* to other computer systems. These computer based ballistic imaging systems allow the Firearms Examiner to quickly review and possibly link large amounts of evidence to a crime while minimizing the evidence chain of custody requirements.

For each of the ballistic imaging systems, the Independent Evaluation Team spent approximately one week (five working days) on-site. These on-site evaluations consisted of demonstrations, real-time *stress tests*, hands-on operational experience, and question and answer sessions. The system stress tests were utilized by the Independent Evaluation Team to gain a better understanding of how each system worked and to determine possible operator bias in data input and sample matching.

The full-up BP system was evaluated at the BATF Forensic Science Laboratory in Rockville, Maryland. The full-up DF system was evaluated at the FBI Laboratory in Washington, DC. During each week of on-site evaluations, the Independent Evaluation Team was accompanied by forensic experts and contractor support from the *outside agency*. The accompanying team helped bring important information to the attention of the Independent Evaluation Team. During each week of systems evaluation, closed door sessions were held to discuss proprietary information. The *outside agencies and contractors* were excluded during these proprietary meetings.

A *controlled baseline database* was developed consisting of five calibers of weapons: 25 Auto, 380 Auto, 9 mm, 38 Special/357 Magnum, and 45 Auto. Each caliber of weapon consisted of thirty (30) distinct guns; two fired bullets and two fired cartridge cases were supplied from each gun. The *baseline database* consisted of a total of one hundred fifty pairs of specimens (5

calibers X 30 guns X 2 specimens each = 300 specimens or 150 pairs). All of these database specimens (including the double blind test specimens, discussed below) were judged by the Firearms Examiners on the Independent Evaluation Team as *minimally damaged or pristine*. The bullets were forwarded directly to BATF and the cartridge cases directly to FBI. This entire set of specimens, including the test specimens, are referred to as the *ONDCP database* throughout this report. A self correlation and a double blind test was conducted on both systems. These tests, based on five calibers of handguns, were designed and overseen by the two Firearms Examiners on the Independent Evaluation Team. For these tests, the databases were delineated by caliber only. Furthermore, the five separate caliber databases were artificially enlarged by adding previously existing images representing individual weapons. That is, the *ONDCP database of bullets* was enlarged by adding images already on file at BATF. Similarly, the *ONDCP database of cartridge cases* was enlarged by adding images already on file at FBI. These additional bullet and cartridge case images were selected at random by BATF and FBI, respectively. These enlarged databases were created to better simulate real-world field operations. Specifically, the following table summarizes the test series database sizes used in these tests. Also note that BATF had a large image database of 38 Special/357 Magnum weapons; therefore, an additional test database was constructed *only* for BP by adding 350 images representing 38 Special/357 Magnum handguns (denoted by an asterisk in the table). BATF, FBI, and OMB agreed to the design of the individual databases.

Database Sizes for BP and DF Test Series

Caliber	Series Designation	ONDCP Supplied Weapons	FBI/BATF Add-on Weapons	Total Weapons in Database
25 Auto	A	30	100	130
380 Auto	B	30	250	280
9 mm Luger	C	30	500	530
38 Sp./357 Mag.	D	30	30 (350*)	60 (380*)
45 Auto	E	30	100	130

The *self correlation test* was performed only on the 9 mm Luger samples (series C, 530 samples total). This test simply asked the ballistic imaging systems to find the respective mate to each of the thirty (30) pairs of ONDCP samples in the expanded 9 mm caliber database. The second test was a *double blind* test series for each of the five calibers. This double blind test series requested the BP and DF systems to determine new matches in the ONDCP database for an additional set of controlled test samples. Specifically, an additional ten (10) test samples per caliber (*i.e.*, 50 bullets and 50 cartridge cases, some of which were control samples) were supplied to BATF and FBI. The BP and DF personnel were asked to conduct their standard computer correlation, selection, video image comparison, and identification process. The result of each of the fifty (50) blind tests was a final list of high confidence candidates, if any. Under normal circumstances, the Firearms Examiner would requisition these high confidence candidates for physical examination under comparison microscope to make a conclusive identification. The results of the double blind tests were based on this list of high confidence candidates. The double blind test results were examined using three (3) statistical techniques to establish the validity of the results. In all three statistical analyses, the test results were found to be significant at the 95% confidence level.

To perform the Life Cycle Cost analysis, the *national scale systems deployment* model was as follows: beginning in FY95, four (4) operational clusters are deployed annually for a total of twenty active clusters by the end of FY99. All clusters are deployed as five site networked systems (for a total of 100 individual computer systems by the end of FY99). Each cluster of five networked systems would consist of one central imaging and analysis station (*i.e.*, the server) and four regional imaging and analysis stations. The central imaging and analysis station acts as the cluster control unit and master database hub; it would also pass data among all units in the cluster. *State and Local Work-Years are not included in this Life Cycle Cost model for either the BP or DF system.*

The findings from this evaluation study show a number of interesting and candid results. The most notable of these results are:

1. Both ballistic imaging systems are extremely useful to the Firearms Examiner in their current configuration.
2. There are approximately 160 Federal, State, and Local Forensic Laboratories in the United States that could benefit from the deployment of one or both of these ballistic imaging systems. The deployment of the BP and DF systems should result in an increase in linking and solving more criminal cases.
3. The deployment of the BP and DF ballistic imaging systems will not reduce manpower requirements.
4. The BP and DF systems are not redundant. However, they perform similar functions on different types of ballistic evidence.
5. The procedure of matching bullets is inherently more complex compared to matching cartridge cases. This result is simply due to the *nature of the evidence* and the amount of data that must be analyzed to perform the image matching task by the computer algorithms. The BP system is addressing the more difficult problem of matching bullets. The DF system is addressing the problem of matching cartridge cases. The Independent Evaluation Team does not know of any other ballistic imaging systems capable of performing these tasks. Also, there is a lack of historical information for computer based ballistic image correlation tests to judge the respective performances of these systems.
6. BP and DF represent major improvements in ballistic identification technologies. To realize the full potential of the systems would require continued engineering development. Both systems have enormous potential to become an extremely effective tool to the Firearms Examiner.

7. A Firearms Examiner requires state-of-the-art data acquisition, image matching, image manipulation, and networked communications capabilities available in modern computer based technologies. Clearly, a Firearms Examiner (or other end user) would be much more efficient and knowledgeable on a single, versatile, state-of-the-art ballistics imaging system.
8. From a systems engineering point of view, the Firearms Examiners' ballistic imaging system requirements can be met by available technology. Based on the current technological status of the BP and DF systems (the only two ballistic imaging systems on the market today), the Firearms Examiners' ballistic imaging system requirements would be met by integrating BP and DF into one common versatile platform. Also quite interesting is that, generally, weak points in one system are strong points in the other. Specifically, the *front end* (microscope, lighting system, and data acquisition system) of the BP system should be combined with the *back end* (computer system and networking capability) of the DF system. Both the BP and DF systems have proprietary operational computer image correlation algorithms which should be used in the common platform.
9. The results of the auto correlation tests showed that BP ranked the test match in the *first place position* 25.6% of the time; DF ranked the test match in the *first place position* 13.3% of the time. For rankings in *positions one (1) through ten (10)*, BP found the test match 42.6% of the time compared to 56.6% for DF.
10. In the double blind tests, BP operators identified 20 of 30 possible correct matches (*i.e., hits*); DF operators identified 28 of 30 possible correct matches. Also, from the 20 control samples (*i.e., test specimens without mates in the database*), BP had four (4) false positives; DF had three (3) false positives.
11. The results of the double blind tests indicate BP would have difficulty identifying smaller caliber bullets.
12. The results of the double blind tests allowed a comparison of the image matching comparison speed. From scanning the test specimen into the database to generation of the final high confidence candidate match list, the DF system established a match at least three (3) times faster than the BP system. If specimen images are already present in the database, the DF system establishes a match least seven (7) times faster than the BP system. This noticeable difference in times to establish a match can be explained by two main considerations. First, BP requires the recording and analysis of megabytes of bullet image data while DF requires only kilobytes of cartridge case image data. Second, a Firearms Examiner simply requires more time to conduct a visual examination on a pair of bullets compared to a pair of cartridge cases.

13. Using the *five year national scale systems deployment* model, the BP system would be approximately three (3) times more expensive than DF to deploy on a national scale, with current pricing under Federal contracts, including volume discounts. Specifically, the results from the model indicate that the BP deployment would require approximately \$41,221,000 and 82 BATF Work-Years; the DF deployment would require approximately \$13,568,000 and 33.5 FBI Work-Years. *State and Local Work-Years are not included in this Life Cycle Cost model for either system.*
14. For single system purchases, the price differential BP and DF expands to range from approximately 6:1 to 10:1, depending on system configurations. Specifically, the current single unit purchase price for a stand-alone SAS/DAS BP system is approximately \$540,000. The single unit purchase price for a baseline stand-alone DF system ranges from approximately \$51,000 (client and server operations on one SPARCstation™) to \$95,000 (client and server operations on two separate SPARCstation™s). These figures, based on current contracts and pricing, include hardware and software procurement, installation, checkout, and initial training. BP systems are offered at discount pricing for quantity purchases.
15. The United States Government should consider performing a *Should Cost Analysis* of an integrated system with the capabilities of both the BP and DF systems before acquiring any ballistic imaging system(s). Through a first order approximation, the Independent Evaluation Team estimates the *Should Cost* of such an integrated system to be in the range of \$150,000 to \$250,000.
16. The United States Government should consider performing a *Cost Benefit Analysis* on such an integrated ballistic imaging system before acquiring any ballistic imaging system(s).
17. Several specific recommendations have been conveyed to the developers of the BP and DF ballistic imaging systems. These recommendations are listed on pages 37 and 38 of this report.

The following pages describe the performance of the BP and DF systems based on the MOEs. These results, and others, are documented and discussed in detail throughout the remainder of this report.

This report represents the opinions of the *entire* Independent Evaluation Team; no Independent Evaluation Team Member offered any dissenting opinion. Both BATF and FBI have supplied addendums to this report which are contained in Appendices F and G, respectively.

BULLETPROOF® and DRUGFIRE™

Performance Chart

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Type of Evidence Analyzed	Bullets	Cartridge Cases
Overall System Accuracy was measured solely on the self correlation and double blind tests.		
a. Self Correlation Results:		
First Place Position	25.9%	13.3%
Positions 1 to 10	42.6%	56.6%
b. Double Blind Test Results:		
True Negatives	16 of 20	17 of 20
False Positives	4 of 20	3 of 20
Hits	20 of 30	28 of 30
Misses	10 of 30	2 of 30
System Processing Speed was measured by the time required for data acquisition and the time for automated search and correlation.		
a. Data acquisition:		
1. Time to calibrate the system for scanning an initial test sample:	No Procedures	No Procedures
2. Time to set up a sample for scanning:	Approx. 2 minutes	Approx. 1 minute
3. Time required to acquire the test sample data, display the data, and verify the data's completeness and accuracy:	Approximately 14 minutes	Approximately 11 minutes
4. Data transfer time:	Approx. 1 minute for 1 bullet image from DAS to SAS on the LAN	Automatic
b. Search and Correlation:		
1. Time required to search the database to obtain primary candidate matches:	Approximately 2 to 7 seconds/image	Approximately 7 to 10 images/second
2. Time required for the Firearms Examiner to view the primary candidate(s) and the reference specimen on the high resolution computer monitor:	Approximately 5 minutes per bullet	Approximately 6 minutes per cartridge case

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
<p>Overall Processing Capability was based on the time required to correlate 1000 unknown specimens against an established database consisting of 1000 images. In this hypothetical measure, the 1000 unknown samples are <i>not</i> added to the database. For both BP and DF, these times scale linearly with database size and number of correlation search requests.</p>		
	20.8 days	1.25 days
<p>Complexity was a qualitative measure to gauge system operational qualities such as calibration, sample preparation, data acquisition, display, processing, data storage, image correlation, and image standards and quality assurance techniques.</p>		
a. Ease of system calibration:	No calibration	No calibration
b. Ease of sample preparation:	Easy, C clamp jig, mounting stub	Easy, Sticky wax, needs mechanical jig
c. Ease of data acquisition, display, processing, and storage:	Operator sets video image boundaries, focus, illumination level; user must view 2 video screens; users can input detailed case file information; users must initiate transfer from DAS and receive on SAS for data storage; system is easy to learn and use.	Operator must adjust specimen centering, focus, illumination, and orientation (rotation); users can input detailed case file information; image storage is automatic and transparent to the user; system is easy to learn and use.
d. Ease of test sample image correlation to the database:	User can select multiple filters based on GRCs and other characteristics for an <i>individual</i> correlation; selection is menu driven and easy to use; <i>batch</i> runs only incorporate system default GRC filter settings.	User can select multiple filters based on GRC filters. Selection is menu driven and easy to use.
e. Image standards requirements - quality assurance techniques:	Performed by user; highly user subjective.	Performed by user; highly user subjective.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
<p>Computer Requirements were measured by a description of system computer(s) capabilities (operating speed, RAM, <i>etc.</i>), demographic data used in the image search and correlation process, the ease of modification and editing of the main database file, and supporting peripheral equipment.</p>		
a. System computer(s) capabilities:	<p>DAS consists of a 486DX2, 66MHz, EISA Bus, 20 MB of RAM, 170 MB hard drive, and 1.2 GB erasable optical disk. SAS is the same, but has an additional 1 GB hard drive and 525 MB cartridge tape.</p>	<p>Client system consists of a Sun SPARCstation 10 with 32 MB of RAM, 1 GB hard drive, and 19" high resolution monitor. Server system is same, with 4 GB hard drive, 250 MB cartridge tape drive.</p>
b. Demographic data used and their effect in the image search and correlation process:	<p>Standard GRC filters and other user specified characteristics to effectively narrow the search space; additional built-in filter based on LEA widths to quickly accept or reject candidate images for correlation.</p>	<p>Standard GRC filters and other user specified characteristics to effectively narrow the search space.</p>
c. Ease of modification and editing of the main database file:	<p>User can only perform modifications from the DAS; modifications require an additional transfer session to effect changes in the database.</p>	<p>Individual case mods can be made from any client; only the database administrator can delete files from the database.</p>
d. Supporting peripheral equipment:	<p>Video and image printer strongly suggested.</p>	<p>Video and image printer strongly suggested.</p>

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Database Size/Restrictions was measured by storage requirements, total number of data sets that can be held in the system data storage component, level of detail captured in the original imaging process and data compression capability and its affect on image resolution and quality.		
a. Storage required for each sample data set:	Approximately 350 kB per LEA (i.e., 2.1 MB for a 6 LEA bullet); uncompressed images are stored on the DAS optical drive; a 2.1 MB original image is re-sampled and compressed to approximately 200 kB; text and signature data are approximately 20 kB per bullet; compressed image data are stored on the SAS optical drive; text, and signature data are stored on the SAS hard drive.	Each standard image size is approximately 30 kB; the user has option of storing multiple standard images and other auxiliary images; single cartridge case data file with 2 standard images and 3 additional images auxiliary images are approximately 150 kB on the server.
b. Total number of data sets that can be held in the system data storage component(s):	600 uncompressed images on the DAS optical disk drive. Approximately 3,000 compressed images on the SAS optical disk drive; approximately 50,000 text and signature data files on the SAS hard drive.	Approximately 27,000 cartridge cases on the server.
c. Data compression capability and its affect on image resolution and quality.	Data compression is a 5:1 JPEG (i.e., 80%). FTI was unable to demonstrate side-by-side viewing of the compressed and original images.	Data compression is a 10:1 JPEG (i.e., 90%). FEs sighted minor differences in the compressed and uncompressed images, but believed the losses to be insignificant.
d. Level of detail captured in the original imaging process:	Adequate	Adequate

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Interface Compatibility was measured by noting bullet or cartridge size limits, interface between the CCD camera and the microscope, hardware requirements adherence to industry standards, and calibration and quality assurance procedures.		
a. Bullet or cartridge size limits:	Only calibers between 25 Autos and 45 Autos were tested.	Same.
b. Interface between the CCD camera and the microscope:	Excellent	Excellent
c. Hardware conformance to industry standards:	Conforms to standards	Conforms to standards
d. Software conformance to industry standards:	Conforms to current industry standards, but the software design and implementation are poor; software includes a <i>closed custom</i> database with no data exchange capabilities; non-multitasking system software.	Conforms to current industry standards. The software design and implementation are well done.
e. CCD camera calibration and quality assurance procedures:	No calibration; no established QA/QC.	No calibration; no established QA/QC.
f. Minimum evidence requirements:	Must be able to define 1 land impression.	Not adequately tested.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Network Compatibility was measured by network robustness, networking hardware requirements, and software and database security requirements.		
a. Network functionality:	Poor. Software requires operator intervention at sending and receiving ends to effect all network transfers; only one network operation is possible at a time.	Excellent. State-of-the-art, real-time video comparison and text dialogs between multiple clients, and e-mail.
b. Networking hardware requirements:	Requires standard 14.4 kB dial-up modem for WAN operations; optional DES.	LAN is built-in. WAN requires an external terminal server, dedicated telephone line, 56 kB modem, optional DES.
c. Software and database security requirements:	Requires Novell NetWare-Light software for LAN and WAN operation; poor security arrangements due to the single user password for all users.	Excellent network security; separate ID and password for each user, system administrator, and database administrator; users are not allowed access to the UNIX™ operating system.
d. System and network administration procedures and backup:	All backups are manually initiated. No other system administration was specified.	Daily backups are automatic. Full system backup requires operator to change tapes. System administrator must add and delete all users. Non-catastrophic failures can be handled over the network.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
<p>Human Factors was a quality measurement of man-machine interface limitations, technician qualifications requirements, technician training requirements, ease of sample processing, specimen handling procedures, operator bias in data acquisition and sample matching, and customer support access.</p>		
a. Man-machine interface limitations:	<p>Operator must re-focus attention between two video screens; GUI is easy and fast to learn and operate; several functions are mouse driven; mouse <i>can not</i> be used during high resolution video image comparison; ergonomics are very good.</p>	<p>Operation on single screen/single station; system is easy to learn and operate; visual and audio prompts; specimen mounting and adjustment procedures are contorted; algorithm score numbers are confusing; small video image size could mask potential matches; video image comparison is excellent; ergonomics are very good.</p>
b. Technician qualifications requirements (expertise, education, manual dexterity, etc.):	<p>Suggested: AA degree, approximately 120 hours of varied classes, computer familiarity, visual acuity, and form perception.</p>	<p>Same</p>
c. Technician training requirements to include formal classes and on-the-job training:	<p>Suggested: DAS operator: approx. 32 hrs of a firearms safety class, 40 hrs of basic firearms identification training, 40 hrs of system training; SAS operator: 20 day training session on the DAS/SAS.</p>	<p>Same level of training.</p>

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Human Factors (continued)		
d. Operator bias in data acquisition:	With the exception of defining LEA widths, the system was reasonably tolerant to operator bias.	With the exception of centering the image, the system was reasonably tolerant to operator bias.
e. Operator bias in sample matching:	Operator can define selection criteria based on image correlation scoring values; scoring data is displayed in tabular form which may lead to operator fatigue & errors.	Operator may be subject to fatigue and eye strain; misaligned or rotated images may cause problems; operator is not adversely affected by the ranking system; images presented in rank order.
f. Customer support access:	Currently adequate; user groups established to help identify future system hardware and software upgrades; user must pay for all hardware and software upgrades; no mechanism exists to insure that all BP cites are running identical versions.	Currently good; user groups established to help identify future system hardware and software upgrades; free software upgrades insuring uniformity throughout the user base; user must pay for commercial software upgrades.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Environmental Limitations was a qualitative measure that took note of special environmental requirements, hazardous materials handling requirements, and hazardous materials disposal requirements.		
a. Special environmental requirements:	May require air conditioning for operator comfort.	Same
b. Hazardous materials handling and disposal requirements:	None	None
Facilities was qualitative measure that noted space requirements, special facilities features requirements, and hardware/system security requirements.		
a. Space requirements:	DAS/SAS require approximately 10' X 15' (150 square feet).	Server and client can fit on a large desktop table approximately 4' X 5' (20 square feet).
b. Special facilities features requirements:	Two (2) separate 115 Volt, 15 Ampere, 3 wire dedicated circuits.	One (1) 115 Volt, 15 Ampere, 3 wire dedicated circuit.
c. Hardware and system security requirements:	To prevent unauthorized hardware and software access, the system must be located in a physically secure room.	To prevent unauthorized hardware access, the system must be located in a physically secure room; no special requirements are needed to protect the DF software.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
<p>Expandability was a qualitative MOE which was measured by type of computer(s) and its ability to accept additional software, automatic image correlation as part of the data acquisition process, ability of system to process other firearms evidence, pre-planned product improvement programs, ability of the system to exchange data and imagery with other sources, ability to accept future hardware updates, and ability to interface with other computer systems.</p>		
a. Ability to accept other software:	Abundance of software is available for PCs, but not compatible the with current configuration of BP software; incorporating new algorithms is feasible, but must be custom integrated with the software.	Abundance of software is available for Sun Workstations; all this software should be compatible with current DF system.
b. Automatic image correlation as part of the data acquisition process:	Not feasible under the current software.	Currently not available, but could be easily implemented.
c. Ability of system to process other firearms evidence:	Hardware is capable of processing other firearms evidence; FTI is developing a cartridge case imaging system named BrassCatcher™ which is scheduled for release in October, 1994.	Hardware and software is capable of processing and storing other types of forensic data. Sybase is SQL compatible allowing the database to be easily modified.
d. Pre-planned product improvement programs:	Re-evaluation of the computer system architecture, including hardware and software, is currently ongoing at FTI; continued development of fully automated data acquisition system, and matching algorithm; planned release of BrassCatcher™, a cartridge case matching system, scheduled for release in late 1994.	Continued development of the matching algorithm; targeted 1 software upgrade release each quarter; product improvement agenda driven by steering committee, user groups, and available funding.

Measure of Effectiveness (MOE)	BULLETPROOF®	DRUGFIRE™
Expandability <i>(continued)</i>		
e. Ability of the system to exchange data and imagery with other sources:	Can not directly exchange data and imagery with other sources.	Can exchange data with other systems through Sybase ASCII flat files and other UNIX™ based commercial software packages.
f. Ability to accept future hardware updates:	Can accept PC hardware and peripheral equipment upgrades; architecture is modular making such hardware upgrades relatively simple; numerous lines of assembly code may require modification for certain upgrades.	Can accept future hardware and peripheral equipment upgrades; Sun SPARCstation™ 10s can readily incorporate multiple processors; system architecture incorporates standard SCSI devices and ports.
g. Ability to interface with other computer systems:	Additional hardware and software is required to access non-DOS systems.	Standard communication is possible with all other system architectures.

Journal Review

Journal of Clinical Forensic Medicine
Churchill Livingstone Journals, Edinburgh, UK
ISSN 1353-1131
Subscription Rate: Variable
(telephone: 027-962-3760 for information)

The *Journal of Clinical Forensic Medicine* is a peer-reviewed journal intended to disseminate information of interest to forensic scientists, forensic pathologists, physicians, medical examiners, and other individuals associated with forensic medicine. Its approach is multidisciplinary, with emphasis on case reports and articles concerning the examination and handling of criminal matters with a forensic medical perspective.

Original articles are presented in a format consistent with traditional peer-reviewed journals. Review articles are commissioned by subject area experts to discuss particular aspects of specific disciplines and offer contrasting viewpoints on controversial issues. The articles are well-referenced and provide clear illustrations.

Case reviews and case reports address pertinent medical and legal questions and offer useful information relevant to situations that are likely to be encountered by forensic medical professionals. Literature citations, book reviews, meeting reports, and a calendar of forthcoming professional meetings and conferences are also featured. Literature citations summarize articles recently published in other forensic journals and are valuable for individuals who may not have access to these journals.

The *Journal of Clinical Forensic Medicine* provides a forum for forensic specialists to share original ideas, research projects and results, and interesting case reviews and case reports in a timely manner. It seeks to become an international vehicle for the discussion of new methods and approaches to dealing with the complex and expanding fields of forensic medicine.

Reviewed by: John E. Mertens
Federal Bureau of Investigation
Washington, DC

Editor's Note

I am very pleased to be joining the staff of the *Crime Laboratory Digest* as the new editor, and I would like to thank Colleen Wade for her valuable contributions while serving as the managing editor. Denise K. Bennett has now assumed the role of managing editor.

Bruce Budowle

CRSE and MPFSL Reports

Copies of the following Central Research and Support Establishment (CRSE) Reports and Metropolitan Police Forensic Science Laboratory (MPFSL) Reports are available for all duly authorized crime laboratories. To obtain copies of the reports, forward a written request to the following address. Supplies are limited, and all requests must be received by July 31, 1995.

Federal Bureau of Investigation
FSIRS Librarian, Room 3790
10th Street and Pennsylvania Avenue, NW
Washington, DC 20535
(telefax: 202-324-4323) (internet: cwade@capcon.net)

Detection of P30 and 19-OH Prostaglandin F1, Specific Human Semen Markers, in the Presence of a Range of Possible Casework Contaminants, by Enzyme Linked Immunosorbent Assay (ELISA): Blind Trial Results
Sutton, J. G.
CRSE Report #760

UK Caucasian Database for TBQ7 (D10S28) Locus Derived from Blood Samples Submitted for Paternity Analysis
McDonald, A. J.
CRSE Report #761

Detection of Linearised Plasmid DNA Using a Fluorescent Dye YOYO-1 in Agarose Gel Electrophoresis
Faulkner, K. L.
CRSE Report #762

Further Observations on Glass Evidence Interpretation
Evetts, I. W.
CRSE Report #763

Review of Methods and Practices for the Enhancement of Footwear Impressions
Allen, T. J.
CRSE Report #764

Harwell Matchfinder: A Chromatographic Profile Matching Program. Part II: The Visual Comparison of Complex Chromatograms and the Use of Matchfinder to Discriminate Between Closely Related Chromatograms
Willson, D.
MPFSL Report #93

Free-Standing Cabinet for Cyanoacrylate Fuming
Stokes, M.
MPFSL Report #94

Arterial Pump: A Device for Simulating Arterial Bleeding
Stokes, M.
MPFSL Report #95

Employment Opportunities

Forensic Examiner (Latent Prints)

Salary Range: \$28,379 – \$39,733 per year

Qualifications and Experience: Applicants must have a bachelor's degree in criminology, administration of justice, or a related field. Certification by the International Association for Identification (IAI) is required, and automated fingerprint identification system (AFIS) training and experience is preferred. An equivalent combination of education and experience may be considered.

CONTACT:

Guy McCormick
City of Wichita
455 North Main Street
Wichita, KS 67202
(telephone: 316-268-4531)

EQUAL OPPORTUNITY EMPLOYER

Questioned Documents Examiner

Salary Range: \$40,768 – \$46,425 per year

Qualifications and Experience: Applicants must have a bachelor's degree in any field plus 2 years of apprenticeship under the tutelage of an American Board of Forensic Document Examiners (ABFDE) or American Society of Questioned Document Examiners (ASQDE) certified examiner and 2 years of independent work experience in a forensic document laboratory. Certification by the ABFDE is also required.

CONTACT:

City of Lakewood (Police Department)
Department of Employee Relations
445 South Allison Parkway
Lakewood, CO 80226
(telephone: 303-987-7700)
(telefax: 303-987-7678)

EQUAL OPPORTUNITY EMPLOYER

Latent Print Examiner II

Salary Range: \$31,803 – \$45,219 per year

Qualifications and Experience: Applicants must have an associate's degree or at least 60 accredited semester hours in criminalistics or a related field plus 3 years of experience involving both inked and latent fingerprint classification work in a law enforcement environment, supplemented by formal training in latent fingerprint, photography, and other police identification techniques. Applicants also must be court-qualified as an expert witness.

Latent Print Examiner III

Salary Range: \$33,530 – \$47,570 per year

Qualifications and Experience: Additional requirements include a bachelor's degree and certification by the International Association for Identification (IAI).

CONTACT:

Frank J. Rodgers
Phoenix Police Department
620 West Washington Street
Phoenix, AZ 85003-2187
(telephone: 602-262-6197)
(telefax: 602-534-4029)

EQUAL OPPORTUNITY EMPLOYER

Criminalist (Trace Evidence)

Salary Range: \$28,008 – \$42,012 per year

Qualifications and Experience: Applicants must have a bachelor's degree from an accredited college or university in chemistry, criminalistics, or forensic science plus 2 years of experience in the examination of trace evidence, including glass, paint, and fibers.

CONTACT:

Frank Shiller
Police Department Crime Laboratory
350 West Belknap Street
Fort Worth, TX 76102
(telephone: 817-877-8084)
(telefax: 817-877-8202)

EQUAL OPPORTUNITY EMPLOYER

Criminalist II (Trace Evidence)

Salary Range: \$37,579 – \$52,892 per year

Qualifications and Experience: Applicants must have a bachelor's degree in chemistry plus 3 years of forensic chemistry work experience which includes the examination of glass, paint, fibers, soils, flammables, and other trace evidence. Applicants must be court-qualified as an expert witness in trace evidence analysis. Knowledge of the rules of evidence is essential.

CONTACT:

Broward County Sheriff's Office
Personnel Division
2601 West Broward Boulevard
Fort Lauderdale, FL 33312
(telephone: 305-321-4400)

EQUAL OPPORTUNITY EMPLOYER

Senior Criminalists (Latent Prints)

Salary Range: \$31,226 – \$41,181 per year
plus benefits

Qualifications and Experience: Applicants must have a bachelor's degree in chemistry, criminalistics, chemical engineering, metallurgy, forensic science, a biological science, or a related field plus 3 years of experience as a laboratory criminalist. An equivalent combination of education and experience may be considered, and some of the six available positions may be under-filled as a Criminalist I or Criminalist II.

CONTACT:

Lois Ray
Oklahoma State Bureau of Investigation
PO Box 11497
Oklahoma City, OK 73136
(telephone: 405-848-6724)
(telefax: 405-842-0675)

EQUAL OPPORTUNITY EMPLOYER

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Meeting Announcements

1995

- May 8-12 **Joint Meeting of the Northwest Association of Forensic Scientists (NWAFS) and the Alaska Peace Officers Association** at the Hilton Hotel in Anchorage, AK. For further information, contact George Taft, Director, Scientific Crime Detection Laboratory, 5500 East Tudor Road, Anchorage, AK 99507 (telephone: 907-269-5740 or telefax: 907-338-6614).
- May 10-12 **Annual Meeting of the Mid-Atlantic Association of Forensic Scientists (MAAFS)** at the Fair Oaks Holiday Inn in Fairfax, VA. For further information, contact Eileen Davis, Virginia Division of Forensic Science, Northern Laboratory, 9797 Braddock Road, #200, Fairfax, VA 22032 (telephone: 703-764-4600 or telefax: 703-764-4633).
- May 10-13 **85th Semi-Annual Seminar of the California Association of Criminalists (CAC)** at the Walnut Creek Marriott Hotel in Walnut Creek, CA. For further information, contact Karen Sheldon, Contra Costa County Sheriff-Coroner's Department, 1122 Escobar Street, Martinez, CA 94553 (telephone: 510-646-2455 or telefax: 510-646-2913).
- June 5-9 **26th Annual Training Seminar of the Association of Firearm and Tool Mark Examiners (AFTE)** at the Bahia Hotel in San Diego, CA. For further information, contact James Roberts, Los Angeles Sheriff's Department, Firearms Identification Unit, 2020 West Beverly Boulevard, Los Angeles, CA 90057 (telephone: 213-974-4628 or telefax: 213-413-7637).
- September 26-30 **42nd Annual Meeting of the Canadian Society of Forensic Science (CSFS)** at the Delta Chelsea Inn in Toronto, Ontario, Canada. For further information, contact Dr. Joel Mayer, Centre of Forensic Sciences, 25 Grosvenor Street, Toronto, Ontario, Canada M7A 2G8 (telephone: 416-314-3159 or telefax: 416-314-3181).
- October 5-7 **Joint Training Conference of the International Association of Bloodstain Pattern Analysts (IABPA) and the Association of Crime Scene Reconstruction (ACSR)** at the Meridian Plaza Hotel in Oklahoma City, OK. For further information, contact Captain Thomas Bevel, Oklahoma City Police Department, 701 Colcord Drive, Oklahoma City, OK 73102 (telephone: 405-297-1225 or telefax: 405-297-1360) or Michael Dixon, Oklahoma State Bureau of Investigation, PO Box 1727, Enid, OK 73702 (telephone: 405-242-2600 or telefax: 405-234-8707).
- October 15-21 **Joint Meeting of the Midwestern Association of Forensic Scientists (MWAFS) and the Southern Association of Forensic Scientists (SAFS)** at JR's Executive Inn in Paducah, KY. For further information, contact Glenn Schubert or Grace Johanson Lively, Southern Illinois Forensic Science Centre, 606 East College Street, Carbondale, IL 62901 (telephone: 618-457-6714 or telefax: 618-457-4676).
- October 16-20 **Fall 1995 Meeting of the Northwest Association of Forensic Scientists (NWAFS)** at the Ashland Hills Inn in Ashland, OR. For further information, contact Wayne Ferguson, National Fish and Wildlife Forensics Laboratory, Serology Section, 1490 East Main Street, Ashland, OR 97520 (telephone: 503-482-4191 or telefax: 503-482-4989).
- October 18-21 **86th Semi-Annual Seminar of the California Association of Criminalists (CAC)** at the Sheraton Harbor Hotel in Los Angeles, CA. For further information, contact Joe Hourigan or Larry Blanton, Los Angeles Police Department Criminalistics Laboratory, 555 Ramirez Street, Space #270, Los Angeles, CA 90012 (telephone: 213-237-0058 or 213-237-0061 or telefax: 213-237-0040).
- October 26-28 **21st Annual Meeting of the Northeastern Association of Forensic Scientists (NEAFS)** at the Mystic Hilton in Mystic, CT. For further information, contact Donald Doller, Suffolk County Crime Laboratory, Suffolk County Office Building #487, Hauppauge, NY 11787 (telephone: 516-853-5585 or telefax: 516-853-5739).

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