

# DNA Analyst Training Laboratory Training Manual

## Protocol 2.01 Quality Assurance



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S  
**DNA**  
INITIATIVE



## **Forensic Biology/DNA Quality Assurance Table of Contents**

- I. Goals and Objectives**
- II. Organization and Management**
- III. DNA Personnel Qualifications and Training**
- IV. Sample Handling and Facility Requirements**
- V. Evidence Control**
- VI. Validations**
- VII. Analytical Procedures**
- VIII. Incident Reports**
- IX. Critical Reagent Quality Control**
- X. Equipment Function Checks and Maintenance**
- XI. Proficiency Testing**
- XII. Audits**
- XIII. DNA Help Desk**
- XIV. Reviews**
- XV. Safety**

## **Forensic Biology/DNA Quality Assurance Goals and Objectives**

### Overall goals:

To provide comprehensive, uniformly accessible, high quality, state of the art forensic biology services to the citizens of the State of Illinois; and,

To ensure the quality of this Forensic Biology/DNA testing.

### Objectives:

To have documented Forensic Biology/DNA procedures which ensure the output of a quality product;

To routinely monitor Forensic Biology/DNA testing; and,

To document the identification and correction of problems with Forensic Biology/DNA testing.

## **Forensic Biology/DNA Quality Assurance Program Organization and Management**

The Forensic Biology/DNA Quality Assurance Program is part of the Laboratory's Quality Assurance Program.

The following topics are addressed in the Laboratory's QA Manual:

- Quality Assurance Program
- Competency Testing
- Proficiency Testing
- Administrative Reviews
- Quality Assurance Reviews
- Mock Trial/Court Appearance Rating
- Forensic Biology/DNA Quality Assurance
- External Proficiency Testing
- Blind Proficiency Testing
- Corrective Action

## **Forensic Biology/DNA Quality Assurance DNA Personnel Qualifications and Training**

### I. Personnel conducting DNA casework

#### A. Prerequisites for DNA training/casework

Prior to assuming casework responsibilities, each analyst must have a bachelor's degree in a natural science or its equivalent. Each analyst must have successfully completed college coursework in the following areas prior to participating in DNA training:

1. Molecular Biology
2. Genetics
3. Biochemistry

To qualify, courses do not have to have these titles, but must cover equivalent material. The Statewide Technical Leader will review the course syllabus or letter from the instructor describing course content to determine if the course work/equivalents meet the prerequisite requirements.

#### B. Training/Qualifying

1. Each individual will complete a formal period of training or evaluation prior to assuming independent casework responsibilities.
  - a. New analysts will complete the documented DNA training program.
  - b. Experienced analysts will have their training program documentation and technical knowledge reviewed and evaluated.
2. The training/qualifying program will be documented in a training file.
  - a. The training coordinator will document the successful completion of the training/qualifying program in a training file. A check list will be maintained summarizing the training.

- b. Upon the completion of the training program, the training file will be sent to the Director of Training or designate.
- c. Upon completion of the training, the Director of Training will provide a copy of the training checklist and a letter confirming the completion of training to the trainee's laboratory director and the Statewide Technical Leader.

C. Experience

All individuals will have worked in a DNA laboratory for a minimum of 6 months prior to assuming independent DNA casework responsibilities.

D. Certification

Final approval for conducting independent casework rests with the Laboratory Administration.

Initial certification is based on a recommendation by a training coordinator prior to beginning independent casework within the Laboratory.

- 1. To be certified in this manner, an individual will do the following:
  - a. Demonstrate the ability to analyze blood and body fluid stains using the appropriate DNA technology;
  - b. Demonstrate the ability to reproduce accurate and precise results;
  - c. Demonstrate the ability to conduct analysis on non-probative cases;
  - d. Demonstrate theoretical knowledge of DNA analysis;
  - e. Demonstrate competency in mixture interpretation;
  - f. Successfully complete competency tests;
  - g. Successfully complete a mock trial;
  - h. Successfully complete supervised casework; and
  - i. Successfully complete an oral board examination.

E. Continuing Education (CE)

- 1. Each analyst must be responsible for keeping abreast of current developments within the field. Each DNA analyst must complete annual continuing education as required by Standard 5 of the FBI DNA Quality Assurance Audit Document. Annual is defined as per calendar year. A minimum of eight documented hours of CE is required for each analyst. Examples of how this may be accomplished include:

- a. Professional organizations and their meetings;
- b. In-service training;
- c. Attendance at formal training courses;
- d. Participation at in-house technical meetings/courses/seminars;
- e. College coursework;
- f. Internet courses (approved by the Statewide Technical Leader).

In addition, documentation that each DNA analyst has reviewed current literature must be kept.

2. The Laboratory Director will provide the opportunity to participate in these activities as outlined in the following directives:
    - a. Tuition Reimbursement
    - b. Society Memberships
    - c. Section Advisory Committees (SAC)
    - d. Attendance at Professional Meetings
    - e. Out-of-State Travel Requests
- II. Technical leadership of the DNA section will be provided and conducted in accordance with Laboratory programs and Quality Assurance Standards for Forensic DNA Testing Laboratories.

The Statewide DNA Technical Leader's duties are as follows:

- A. Responsible for managing the technical issues and technical problem solving of analytical methods for DNA laboratories to include:
  1. Assistance on difficult or non-routine cases and resolution of disagreements between analysts.
  2. When requested, provides assistance in court preparation, preparation of affidavits in response to special case issues, and testifies as needed, to include Frye hearings.
  3. Serving as the technical point of contact for the outsourcing laboratory to assist with technical questions and issues.
- B. Responsible for documenting an annual review of all methods used by the FSC to include:
  1. The approval of all procedures used in DNA analysis.

- C. Responsible for proposing new or modified analytical procedures to be included in the FSC DNA Procedures Manual to include:
  - 1. The review and approval of all completed Research and Development projects that will affect casework.
  - 2. The proposal of new methodologies or modified analytical procedures to be used by analysts.
  
- D. Responsible for documenting an annual review of the DNA QA Manual and is responsible for the FSC DNA quality assurance program to include:
  - 1. The review of incident reports and assistance with corrective action as needed, including the resolution and reporting of any contamination or extraneous DNA issues identified in a case or lab.
  - 2. The accountability for the lab's quality assurance program to the extent that he or she has the authority to terminate the lab's DNA testing in the event of a technical problem until the problem is solved.
  - 3. Assistance and resolution of statewide DNA QA issues.
  - 4. The resolution of outsourcing quality issues.
  - 5. The maintenance of the DNA analyst/technician/intern database key.
  
- E. Responsible for the documented annual review of each regional laboratory to include:
  - 1. One on-site visit to each laboratory per year. The SWTL will customarily participate in the annual internal audit. (Note: the internal audit will count as the one on-site visit per year.)
  - 2. A review of each laboratory's annual DNA audit. The SWTL will provide input on technical matters which arise from audits, and assist with responses to any audit findings.
  - 3. Additional site visits as needed.

- F. Has oversight responsibility for and will document an annual review of the DNA training and safety programs to include:
    - 1. The oversight of training of laboratory DNA staff.
    - 2. The review of educational qualifications for DNA analysts.
  - G. Responsible for oversight of the FSC DNA proficiency testing program to include:
    - 1. The oversight of proficiency testing performance of analysts via information provided by the Director of Quality Assurance and is responsible for any corrective action.
  - H. Accessible to the laboratory to provide onsite, telephonic or electronic consultation as needed to include:
    - 1. Accurate and timely communication to the DNA analysts regarding Laboratory decisions affecting DNA.
- III. Duties of the members of the DNA Help Desk are as follows:
- A. Assist with questions concerning DNA casework.
  - B. Assist with questions concerning the DNA procedures manual.
  - C. Provide information to the section on technical issues.
  - D. Recommend changes to the procedures manual, when needed.
- IV. The DNA Training Coordinator's duties are as follows:
- A. Design and maintain the Laboratory's DNA training program. Ensure review, input and approval by the Statewide Technical Leader.
  - B. Deliver training to new employees assigned to the Forensic Biology/DNA section, and provide cross-training to existing employees when required.
  - C. Create, administer, and grade written and practical examinations.
  - D. Review all supervised casework generated by trainees.
  - E. Report to the Statewide Technical Leader and Statewide Training Program management any contamination or extraneous DNA issue identified during supervised casework.
  - F. Ensure that all quality control requirements/quality assurance guidelines are followed by individuals in training.
  - G. Monitor court testimony of trainees by actual viewing of testimony.

- H. Lend assistance to analysts in court preparation.
  - I. Give input on technical matters which arise from audits and ensure compliance with DAB standards.
  - J. Provide accurate and timely communications to DNA trainees regarding decisions affecting DNA analysis.
  - K. Work with the Statewide Technical Leader on any other appropriate training issues, such as difficulty with a given aspect of the training program, failure of a criterion test by a trainee, etc.
  - L. Review internal proficiency tests by taking the proposed annual test to determine its validity.
- V. The Local CODIS Administrator/Manager must be a qualified or formerly qualified DNA analyst and must have an understanding of DNA profile interpretation. The CODIS Manager will meet all DNA analyst requirements if they will be performing casework. If they will not be performing casework, they must meet the requirements for a databasing analyst. Local CODIS Administrator/Manager Duties:
- A. Oversees the laboratory's CODIS network.
  - B. Is responsible for backing up data and properly storing the back up media.
  - C. Is responsible for uploading appropriate profiles to SDIS.
  - D. Provides assistance with CODIS computer training of other laboratory staff.
  - E. Requests verification of offender samples and confirmation of conviction matches from SDIS.
  - F. Provides the State CODIS Administrator when appropriate with:
    - 1. Information on CODIS matches
    - 2. User and laboratory changes
    - 3. Requests for NDIS keyboard searches
    - 4. Requests of non-NDIS participating laboratories in the state to have data searched at GDIS, SDIS or NDIS
    - 5. Requests for searches of foreign databases
    - 6. Requests for removal of data at SDIS
  - G. Has the authority to terminate the laboratory's or an individual's participation in CODIS in the event of a problem until the reliability of the computer data can be investigated and assured by the Statewide DNA Technical Leader and the Director of Quality Assurance.
  - H. Oversees the entry and evaluation of outsourcing data and related hits.

VI. Databasing analysts

Databasing analysts will meet all DNA analysts requirements except for non-probative casework, supervised casework and mock trials.

## VII. Laboratory Technicians

- A. Will have documented training, education and experience commensurate with their responsibilities as outlined in job description;
- B. Will perform duties commensurate with their level of training such as bleaching, calibrations, reagent preparation, maintenance of capillary electrophoresis equipment, QA/QC testing and dry down of DNA samples.
- C. Will conduct analytical tests such as Yield Gels and Slot Blots with evidence samples when properly trained and proficiency tested.
- D. Will receive evidence for DNA analysis in accordance with the Evidence Control Policy and the DNA case acceptance policy outlined in the Laboratory Directives; and,
- E. Will return DNA evidence in accordance with the Evidence Control Policy.

## **Forensic Biology/DNA Quality Assurance Sample Handling and Facility Requirements**

- I. All forensic biology and DNA analysts will follow clean technique as documented in the Forensic Biology/DNA procedures manual.
- II. Laboratories conducting DNA analyses will conduct the following activities either in a separate space or at a separate time:
  - Evidence examination;
  - DNA extractions; and,
  - PCR set up.
- III. All processing of unknowns and standards from a case must be separated by time or space. This includes screening, extraction, quantitation, amplification, and 310 sample set-up.
- IV. Amplified products will be contained in a room separate from non-amplified product. Amplified product may be removed from the amplification area for return to the submitting agency or disposal only. If amplified product is removed from the amplification area it will be sealed in a closed container.
- V. Cleaning and Sterilization Procedures
  - A. Appropriate glassware and plastic containers will be cleaned with detergent and completely rinsed with tap water by hand or using a dishwasher. Before laboratory use, these items will be rinsed with distilled water or equivalent. Items that come in contact with DNA samples will be cleaned with detergent and 10% bleach solution, followed by a rinse with distilled water or equivalent.
  - B. The reagent preparation section details those reagents which require sterilization by autoclaving.
  - C. The laboratory will follow the decontamination procedures outlined in the Clean Technique section. A bleach log is used to monitor decontamination of facilities and equipment and will include the items below:
    1. The Forensic Biology/DNA laboratory floor will be mopped using a freshly prepared 10% bleach solution once a week. The PCR room must be mopped last. This must be documented in the bleach log.
    2. The entire Forensic Biology/DNA laboratory (computer tops, equipment, etc.), including the PCR room, must be bleached once a week with a freshly prepared 10% bleach solution. This must be documented in the bleach log.

- D. All waste from the PCR room will be sealed in a closed container before being removed from the PCR room.

## **Forensic Biology/DNA Quality Assurance Evidence Control**

- I. The laboratory system has a documented evidence control system to ensure the integrity of physical evidence. This is outlined in the following Laboratory directives:

- Evidence Receipt Forms
- Submission of Physical Evidence by Mail
- Submission of Forensic Biology Evidence
- Blood Evidence
- Submission of Evidence to the FBI
- Collection of Biological Standards
- Access to Physical Evidence
- Evidence Packaging
- Transferring Cases Between Laboratories
- Case Tracking
- Destruction of Physical Evidence
- Documentation of Case Related Phone Calls or Conversations
- Signature Requirements for Case Reports
- Minimum Standards for Evidence Marking
- Internal Evidence Chain
- Case Acceptance Policy for DNA Analysis
- Clean Technique
- Uniform Guidelines for Mailing Evidence

- II. Biological Evidence Retention and Return Policy
- A. All items of evidence including parent exhibits, stains and extracted DNA must be returned after analysis is completed.
  - B. Extracted DNA (and blanks) from questioned stains remaining after analysis must be dried down in a vacuum centrifuge and returned to the agency.
  - C. Extracted DNA (and blanks) from standards may be discarded if the original sample (stain) was not consumed in analysis.
  - D. Membranes used to obtain RFLP profiles are considered documents and will be retained frozen indefinitely.
  - E. Amplified DNA may be discarded if extracted DNA and/or the evidence samples were not consumed in analysis. Amplified DNA from evidence samples that were consumed in analysis must be double packaged in plastic, and returned to the agency. The package must be labeled "Amplified DNA, Do Not Open."

## **Forensic Biology/DNA Quality Assurance Validations**

- I. The laboratory will use validated methods and procedures as outlined in the Laboratory Directives and, for DNA analysis, meet the requirements listed for standard 8 of the FBI DNA Quality Assurance Audit Document.
  - A. Original copies of all validation study materials will be maintained at the FSC Research and Development Laboratory. Electronic copies will be distributed to each regional laboratory.
  - B. Internal validation studies conducted in the regional laboratories as part of the qualification of a new DNA procedure will be reviewed by the Statewide Technical Leader during the laboratories' internal audit. These studies will be maintained at the individual laboratories.
  
- II.

## **Forensic Biology/DNA Quality Assurance Analytical Procedures**

### **I. Procedures.**

The laboratory will have approved, written analytical procedures.

- A. Procedures used in DNA and Forensic Biology Analysis will be approved according to the Laboratory Directives.
- B. Procedures being developed as part of the R&D Program may be used in casework with Laboratory approval.

### **II. Reagents.**

The laboratory will use reagents that are suitable for the methods employed.

- A. The laboratory will maintain a log for documenting commercial biological reagents and chemicals utilized in the laboratory.
  - 1. Information contained in the log will include the manufacturer, the date a chemical or reagent was received, the lot numbers received, and the quantity received. The expiration date will also be recorded when appropriate.
  - 2. An annual inventory of these reagents will be conducted.
- B. The laboratory will maintain a log for documenting the preparation of all reagents used in the laboratory.
  - 1. The formulas for all reagents are found in the Procedures Manual.
  - 2. Information kept in the log must include the date the reagent was prepared, the lot numbers of chemicals used to prepare the reagent, the quantity prepared, and the identity of the person preparing the reagent.
- C. Reagents will be labeled with the identity of the reagent, the date of preparation, and the initials of the individual that prepared the reagent.
- D. Distilled, deionized water or its equivalent must be autoclaved for use in dilutions for extractions and amplifications.
- E. Expiration Dates

1. Chemicals will expire according to the manufacturer's listed expiration date, if any.
2. Reagents (mixtures of chemicals)
  - a. Purchased reagents will expire according to the manufacturer's listed expiration date. If there is no expiration date, then the expiration date will be one year from the date of receipt at the laboratory.
  - b. Reagents made in the laboratory will expire one year or less from the date of preparation, except for 1% agarose with ethidium bromide which has an expiration date of one month.
  - c. Frozen reagents will have an expiration date of one year from the date they are thawed.
  - d. The following products purchased from Applied Biosystems will expire one year from the date of receipt at the laboratory: ROX, formamide, and matrix standards.
3. Autoclaved distilled deionized water (or its equivalent) used in dilutions for extractions and amplifications will expire one year from the date of autoclaving.
4. Dyes, or mixtures of dyes and water, are not considered reagents and will not have expiration dates.

F. Critical Reagents.

The following have been defined as critical reagents:

Species testing antisera  
Nylon membrane  
DNA amplification kits

G. Critical Reagent Quality Control.

1. Procedures for quality control of these reagents are found in Appendix IV.
2. A critical reagent log will be maintained documenting all quality control procedures performed on a particular lot of a reagent.

3. If a particular supply, chemical, reagent or material does not meet the required quality control standard(s), the manufacturer will be notified and the entire lot rejected.
4. The quality control procedures for critical reagents do not have to be run individually but may be combined with other procedures as appropriate.
5. Quality control records will be maintained indefinitely.

### III. Basic Forensic Biology Procedures:

#### A. Blood Standards

Dry stain cards will be produced from all whole blood specimens as soon after receipt as possible.

#### B. Dry Blood

The minimum work on stains will be to indicate blood. Classifying the stain as human material may be conducted when appropriate.

#### C. Semen

Semen will be identified by the presence of spermatozoa. Examination for the presence of spermatozoa includes observation of either intact or identifiable heads where acrosomal cap, point of attachment, size and shape are clearly visible. If the examination for spermatozoa is negative or inconclusive, then the P30 test will be conducted on the sample, before a failure to identify semen is reported.

Test all vaginal, oral and rectal swabs using the acid phosphatase test.

Examination of clothing items, including underwear, will be conducted if determined to be of probative value. Otherwise clothing will not be routinely examined.

Once semen is identified in a case, the analyst will routinely defer all other semen testing, pending DNA results.

Samples with semen indicated in/on them as a result of positive AP and/or P30 testing may be submitted for DNA analysis at the analyst's discretion.

D. Vaginal secretion will be indicated by the identification of glycogen-containing squamous epithelial cells (positive Lugol's stain test). This test is not confirmatory for vaginal secretion.

E. Saliva

Saliva will be indicated by a positive amylase test. (Phadebas or radial gel diffusion technique.) This is not confirmatory for saliva.

F. Urine

Urine will be indicated by a positive urea nitrogen test or a positive creatinine test.

#### IV. Standard and Controls

The laboratory will monitor the analytical procedures using appropriate controls and standards.

A. The following standards and controls will be used in Forensic Biology casework:

##### 1. Dry Blood - Stain Identification

- a. Kastle-Meyer- a positive and negative control each day the test is used.
- b. Ouchterlony Immunological Tests - a positive and negative control each run.

Test all antisera (human and animal) against the following series of known standards when a new lot is received:

Human, swine, bovine, deer, goat, cat, dog, sheep, chicken or duck (bird), rat or hamster or rabbit (rodent).

Antisera testing will be documented in a logbook. Logbook information will include antisera lot numbers and brand names. Documentation of manufacturer's recommended expiration dates will also be included in the logbook.

2. Semen Identification

Acid phosphatase - two-step procedure. Run known semen and negative control with each set of tubes opened each day.  
P30 by ABACard - run 10ng and 4ng P30 standards and a negative control (blank) with each new lot number of cards. Results are to be recorded in a logbook. Run a negative control (blank) with each daily batch of samples - record this result in the case file.

3. Lugol's Stain

Run known vaginal squamous epithelial cells as a positive control. Run known buccal squamous epithelial cells as a negative control. Run controls each day the test is used.

4. Amylase (Phadebas tablets)

Run known dry saliva extract as positive control and a control blank each day the test is used.

5. Amylase (radial gel diffusion method)

Run a positive control consisting of an aqueous dilution of 1/500 of fresh liquid saliva and a control blank, each day the test is used.

6. Urea Nitrogen

Run known dry urine extract, a control blank, and a portion of the suspected urine stain with the phenol, hypochlorite and nitroprusside without the urease added each day the test is run.

7. Creatinine Test

Known urine stain and a control blank.

B. The following standards and controls will be used in STR casework. These standards and controls must work properly.

1. Positive and negative amplification controls.
2. Allelic ladder.
3. Manipulation blanks. (Refer to [pdi\\_lab\\_pro\\_2.02](#), Clean Technique.)

- C. Compromised results for PCR standards and controls will be handled as follows:
  - 1. If a DNA profile is confirmed in any manipulation blank or negative control, the case must be brought to the attention of the Statewide Technical Leader.
  - 2. If insufficient sample remains for the reamplification or re-extraction of a probative sample, an incident report will be filed.
  - 3. If the DNA profile detected in the manipulation blank or negative control matches the DNA profile of a laboratory employee, interpretation of probative samples will be conducted and reported. (Refer to [pdi lab pro 8.02](#), DNA Report Wording.)
  
- V. The laboratory will have written general guidelines for interpretation of data.
  - A. The laboratory will verify that all control results are typed correctly.
  - B. For a given population(s) and/or hypothesis of relatedness, the probability of observing a DNA profile will be estimated using a standard population genetic method(s) and/or directed method (as described in Forensic Biology/DNA Procedures Manual). These calculations will be derived from a documented population database appropriate for the calculation.

## **Forensic Biology/DNA Quality Assurance Incident Reports**

An Incident Report is used for reporting and resolving incidents that could adversely affect the outcome of a DNA analysis. This includes incidents of the loss of sample or data, contamination, sample switching, etc. A representation of the report form follows. This form is available electronically, and can be forwarded via e-mail.

1. The analyst will notify their supervisor of an incident and complete Part A of the Incident Report form.
2. The supervisor will acknowledge receipt of the form by initialing and dating Part A. He/she will forward a copy to the Statewide Technical Leader and the Director of Quality Assurance.
3. The Statewide Technical Leader will determine a recommended course of action. Part B of the form will be completed.
4. The analyst will follow the recommended course of action
5. The results will be documented on the form with the analyst's and supervisor's initials and date in Part C. The form will be forwarded to the Statewide Technical Leader, along with any necessary data.
6. The Statewide Technical Leader will review the data, determine any follow-up action and document this in Part D of the form. The form will be forwarded to the analyst, his or her supervisor, and the Director of Quality Assurance.
7. The completed form will be maintained in the originating laboratory.

### **Management Responsibilities:**

1. Upon initial notification of the incident, the Director of Quality Assurance in conjunction with the appropriate Bureau Chief, will determine if a situation report or Quality Issue Tracking (QIT) form is necessary.

A copy of the Incident Reports needing quality issue tracking will be maintained by the Director of Quality Assurance as part of the QIT documentation. Those not requiring quality issue tracking will only be maintained by the originating laboratory.

2. Incident reports will be monitored by the Statewide DNA Technical Leader.

**Forensic Biology/DNA Quality Assurance  
Critical Reagent Quality Control of Species Antisera**

1. Purpose: To compare new lot numbers of antisera (human and animal) to the appropriate series of known standards which are human, swine, bovine, deer, goat, cat, dog, sheep, chicken or duck (bird), rat or hamster or rabbit (rodent) as outlined in the Forensic Biology/DNA Procedures Manual.

2. Procedure:

A. Punch the gel with a series of 7 wells to form a hexagon with a central well.

B. Place the antiserum to be QC'd in the central well and bloodstains for 6 different species in the surrounding wells.

C. Cover the petri dish and leave undisturbed at room temperature overnight. The petri dish can be placed in a 37°C oven to decrease the incubation period or in the refrigerator for a longer incubation period.

D. Any known stain which forms a precipitin band with the antiserum must be checked using the following triangular 3-well Ouchterlony pattern:

Set up a positive control: known blood sample in both left and right wells of the triad.

Set up a negative control: known blood sample in one well and a negative (blank) in the other. Place the antisera being QC'd in the third well.

E. Cover the petri dish and leave undisturbed overnight at room temperature. The petri dish can be placed in a 37°C oven to decrease the incubation period or in the refrigerator for a longer incubation period.

F. Record the results on an Ouchterlony worksheet.

3. Assessment of Results:

Precipitin bands which form a continuous arc of convergence (identity) between the antiserum and the two extract wells are considered positive results.

If no or partial precipitin bands form and a positive test result is expected, repeat test. If a positive result is noted for a species other than what antisera it is directed against, repeat test.

If after repeating the test, the results do not coincide with the expectations of the test, do not use that lot antiserum for casework. Notify the manufacturer.



**Forensic Biology/DNA Quality Assurance  
Verification of Charged Membrane for Slot Blot**

1. Purpose: To evaluate a new lot of charged membrane for binding ability and band intensity.
2. Procedure:
  - A. Conduct slot blot analysis as outlined in the Forensic Biology/DNA procedures manual with new lot number of charged membrane. Run slot blot standards and calibrators 1 and 2.
3. Assessment of Results:
  - Compare intensity of signal of standards to calibrators. If band intensity is weak, reject lot number and notify manufacturer.
  - Compare results to the results of previously QC'd membrane. If there is a weaker signal, reject the new lot of membrane.

**Forensic Biology/DNA Quality Assurance  
Charged Membrane  
(For Slot Blot)**

**Manufacturer** \_\_\_\_\_

**Date Received** \_\_\_\_\_

**Lot #'s Received** \_\_\_\_\_

**Quantity Received** \_\_\_\_\_

**Expiration Date** \_\_\_\_\_

**Date QC'd** \_\_\_\_\_

**QC'd by** \_\_\_\_\_

<b>Standards (ng)</b>	<b>Calibrators</b>				

**NOTES:**

**Is band intensity adequate when compared to calibrators?** \_\_\_\_\_

**Is band intensity consistent with that of previously quality controlled membrane?** \_\_\_\_\_

**ATTACH PICTURE OF SLOT BLOT**

**Forensic Biology/DNA Quality Assurance  
Critical Reagent Quality Control of STR Typing Kits**

1. Purpose: To demonstrate that all amplification components contained in the kit can produce accurate typing results.
2. Procedures:
  - A. Prepare amplification reaction mixture using components from new lot number kit, as outlined in the Amplifications section.
  - B. Controls consist of the Control DNA 9947A (present in the kit) and a negative control consisting of 20  $\mu$ l ddi H<sub>2</sub>O.
  - C. Amplify for appropriate loci.
  - D. Type the amplified products.
3. Assessment of Results
  - A. All control samples must type correctly.
  - B. If incorrect or incomplete typing results are obtained reject the lot number and notify the manufacturer.

**Forensic Biology/DNA Quality Assurance  
Verification of COfiler Kit**

Analyst \_\_\_\_\_

QC Date \_\_\_\_\_

Kit Lot # \_\_\_\_\_

Kit Expiration Date \_\_\_\_\_

# of Kits Received \_\_\_\_\_

Date Received \_\_\_\_\_

**Individual Kit Components**

**Please check and Note if Individual Component Lot numbers Vary From Kit to Kit**

Primer Set# \_\_\_\_\_

AmpliTaq Gold # \_\_\_\_\_

Reaction Mix# \_\_\_\_\_

Mineral Oil # \_\_\_\_\_

Allelic Ladder \_\_\_\_\_

9947A# \_\_\_\_\_

	9947A Control DNA	Negative Control
D3S1358		
D16S539		
TH01		
TPOX		
CSF1PO		
Amelogenin		
D7S820		

**NOTES:**

Do all Controls exhibit expected alleles? \_\_\_\_\_

**Include positive control electropherogram and negative control electropherogram along with this sheet.**

**Forensic Biology/DNA Quality Assurance  
Verification of Profiler Plus Kit**

Analyst \_\_\_\_\_

QC Date \_\_\_\_\_

Kit Lot # \_\_\_\_\_

Kit Expiration Date \_\_\_\_\_

# of Kits Received \_\_\_\_\_

Date Received \_\_\_\_\_

**Individual Kit Components**

Please check and Note if Individual Component Lot numbers Vary From Kit to Kit

Primer Set# \_\_\_\_\_

AmpliTaq Gold # \_\_\_\_\_

Reaction Mix# \_\_\_\_\_

Mineral Oil # \_\_\_\_\_

Allelic Ladder \_\_\_\_\_

9947A# \_\_\_\_\_

	9947A Control DNA	Negative Control
D3S1358		
vWA		
FGA		
Amelogenin		
D8S1179		
D21S11		
D18S51		
D5S818		
D13S317		
D7S820		

**NOTES:**

Do all Controls exhibit expected alleles? \_\_\_\_\_

Include positive control electropherogram and negative control electropherogram along with this sheet.

## **Forensic Biology/DNA Quality Assurance Annual System Verification with SRM**

### **Purpose:**

To annually verify that the entire PCR system is functioning within accepted criteria by the use of a standard that is traceable to the NIST Standard Reference Material (SRM). The NIST SRM is a certified standard reference material issued under the NIST trademark. The documentation of the traceability of the standard to the NIST SRM is located at the Indexing Laboratory. The Internal NIST Traceable SRM will be assigned a lot number designated by the date the traceability was established by Indexing and will have an expiration date of ten years.

### **Procedure:**

- A. Extract as per Laboratory protocol.
- B. Amplify using Laboratory protocol for appropriate loci.
- C. Analyze samples using the ABI 310 Genetic Analyzer Capillary Electrophoresis Instrument.

**Assess Results:** Compare the results to the known results for the standard. Any discrepancies will be reanalyzed and resolved. Record the results of the verification on PCR System Form.

**Forensic Biology/DNA Quality Assurance  
Verification of SRM System**

**NIST Traceable Control (SRM) for Year \_\_\_\_\_**

**Analyst:** \_\_\_\_\_

**Date of Analysis:** \_\_\_\_\_

**Include all worksheets and electropherograms along with this sheet. Results:**

	<b>NIST Traceable DNA</b>	<b>Results</b>
<b>D3S1358</b>	<b>16, 18</b>	
<b>vWA</b>	<b>14, 18</b>	
<b>FGA</b>	<b>21, 22</b>	
<b>Amelogenin</b>	<b>X, Y</b>	
<b>D8S1179</b>	<b>12, 12</b>	
<b>D21S11</b>	<b>32, 32.2</b>	
<b>D18S51</b>	<b>12, 13</b>	
<b>D5S818</b>	<b>11, 12</b>	
<b>D13S317</b>	<b>9, 12</b>	
<b>D7S820</b>	<b>8, 10</b>	
<b>D16S539</b>	<b>10, 13</b>	
<b>TH01</b>	<b>7, 9.3</b>	
<b>TPOX</b>	<b>9, 10</b>	
<b>CSF1PO</b>	<b>10, 11</b>	

**Notes:**

**Are all appropriate alleles present?** \_\_\_\_\_

**Are any extra alleles present?** \_\_\_\_\_

## Forensic Biology/DNA Quality Assurance Evaluation of a New ABI 310 Genetic Analyzer

1. When a new Applied Biosystems 310 Capillary Electrophoresis unit is received into the laboratory, it must be evaluated before it can be used for case work. The following studies must be conducted.

When major equipment changes are necessary for the Applied Biosystems 310 Capillary Electrophoresis unit, one or more of the following studies may be required. The type of studies required will be dependent on the type of equipment replaced.

### A. Matrix

Prepare a matrix file using the 5-FAM, JOE, NED, and ROX matrix standards samples and the Filter Set F module file according to the manufacturer's recommendations.

### B. Instrument Run Time

Before proceeding with the following studies the instrument electrophoresis run time must be determined. Run five allelic ladders containing the ROX-500 in-lane sizing standard. Ensure that the 400 base pair peak of the ROX-500 in-lane sizing standard is consistently being observed. If the 400 base pair peak is consistently observed then leave the run time at 24 minutes. If the 400 base pair peak is **not** consistently being observed then increase the run time by one minute and run five more allelic ladders to determine if 400 base pair peak is now being seen.

### C. Precision and Reproducibility

Choose five samples that have been previously characterized using the AmpF/STR system. **Each** sample is to be run 20 times on the ABI 310 CE and analyzed using the GeneScan and GenoTyper software according to Laboratory protocol. Determine the average, standard deviation, minimum, and maximum values for the alleles at each locus for **each** sample. This part of the precision study will also serve as a reproducibility study.

### D. Sensitivity

Choose three samples previously characterized using the AmpF/STR Profiler Plus system and make a dilution series of **each** sample from 5 ng to 0.03 ng. These three samples should contain alleles that represent both high and low alleles across all loci.

**Note:** The data from each of these studies must be maintained in a notebook that is kept near each instrument along with a routine maintenance log.

**Forensic Biology/DNA Quality Assurance  
Annual Verification for the ABI 310 Genetic Analyzer**

A. Matrix:

Prepare a matrix file using the 5-FAM, JOE, NED, and ROX matrix standards samples and the Filter Set F module file according to the manufacturer's recommendations.

B. Sensitivity:

Make a dilution series of the 9947 amplification positive control from 1 ng to 0.03 ng/20  $\mu$ l. Analyze these samples using Profiler Plus.

<b>Verification Study</b>	<b>Analyst Initials</b>	<b>Date Completed</b>	<b>Initials of Technical Reviewer</b>
<b>Matrix</b>			
<b>Sensitivity</b>			

## **Forensic Biology/DNA Quality Assurance Equipment Function Checks and Maintenance**

- I. Calibration Checks:
  - A. For each piece of equipment requiring calibration or checking, the following information will be listed:
    - 1. Procedure
    - 2. Frequency
    - 3. Results
    - 4. Course of action
  - B. Records will be maintained for calibration checks.
- II. Maintenance
  - A. Where appropriate, maintenance procedures and schedules will be listed for equipment.
  - B. Records will be maintained for maintenance

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Balances**

I. Certification

- A. Procedure: Balances will be maintained by a company qualified to provide certification for the balances' accuracy.
- B. Frequency: annually.
- C. Results: The results of the certification (certificate) will be maintained in the laboratory.
- D. Course of action: If a balance fails certification, discontinue its use.

II. Calibration Checks

- A. Procedure:
  - 1. Use standard weights. Handle them with cotton gloves or forceps.
  - 2. Check a minimum of three weights that represent the high, low and medium weights measured on the balance.
- B. Frequency: Check calibration monthly, or if used less frequently, prior to use.
- C. Results: Record the results in a log book. Computerized records will be backed up.
- D. Course of action: If the weights are not within the specified tolerance window, then contact a repair company.

III. Maintenance: No regular maintenance is recommended by the manufacturer.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Oven Temperature Checks**

- I. Temperature Checks
  - A. Procedure: Record the temperature of the oven using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.
  - B. Frequency: Check annually, but observe temperature before each use.
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of action: If the temperature is not within the specified tolerance window, contact a repair company.
- II. Maintenance: No regular maintenance is required.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Refrigerators and Freezers**

- I. Temperature Checks
  - A. Procedure: Record the temperature of the refrigerator or freezer using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.
  - B. Frequency: Check monthly.
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of action: If the temperatures cannot be maintained within the specified tolerance window, contact a repair company.
- II. Maintenance: No regular maintenance is required. Defrost and clean as necessary. Contact the repair company for repairs.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
pH Meter**

I. Calibration

- A. Procedure: Check the calibration with two buffers, above and below the desired pH. Refer to the manufacturer's instruction manual.
- B. Frequency: Check before each use.
- C. Results: For reagents with the pH noted in the formula, the analyst's initials in the reagent log book signifies the calibration checks of the pH meter.
- D. Course of action: If the pH meter does not meet the proper parameters, contact a repair company.

II. Maintenance: No regular maintenance is required.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Pipettes**

I. Calibration Checks

A. Procedure:

1. All pipettes will be checked by a company qualified to provide certification for their accuracy.
2. Each pipette must be checked at three settings that are at the low, middle and high levels in its range of use.

B. Frequency: annually.

C. Results: All three settings must be within the tolerance window of  $\pm 2.5\%$  for each pipette. Record the results in a log book. Computerized records will be backed up.

D. Course of Action: Any pipette whose measurements are outside of the tolerance window must be removed from casework. Submit the pipette for repair and recalibration.

II. Maintenance: No regular maintenance is required.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Microscopes**

- I. Checks
  - A. Procedure: Microscopes will be cleaned and checked by a qualified professional. Optimum illumination will be established, where applicable.
  - B. Frequency: Annually
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of action: If problems are identified, contact a repair company.
- II. Maintenance: Refer to the Microscopy Procedures Manual
  - A. Clean microscopes as necessary.
  - B. Cover all microscopes after use.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Alternate Light Source**

I. Checks

- A. Procedure: Visually examine a 1:50 dilution of semen. This will be prepared by the QA Coordinator every three years.)
- B. Frequency: Check monthly. If used less frequently, check before each use.
- C. Results: Record the results in a log book. Computerized records will be backed up.
- D. Course of action: the dilution is not visualized, make a new stain and retest. If it is still not visualized, contact a repair company.

II. Maintenance: No regular maintenance is required.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Thermal Cyclers**

- I. Temperature Verification and Temperature Uniformity Tests
  - A. Procedure: Using the Temperature Verification System, follow the procedure within the Thermal Cycler User's Guide for the test being conducted.
  - B. Frequency: Conduct Temperature Verification monthly.  
Conduct Temperature Uniformity every 6 months.
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of action: If any temperature falls outside of the documented range, discontinue use. Contact a repair company.
  
- II. Temperature Verification System
  - A. Procedure: Send to a company qualified to provide certification for calibration.
  - B. Frequency: Calibrate annually.
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of Action: If the system fails calibration, discontinue its use.
  
- III. Maintenance: No regular maintenance is required.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Water Baths**

- I. Temperature Checks
  - A. Procedure: Record the temperature of the water in the bath using a NIST traceable thermometer. Adjust the temperature dial if necessary and recheck.
  - B. Frequency: Check monthly.
  - C. Results: Record the results in a log book. Computerized records will be backed up.
  - D. Course of action: If the temperature cannot be maintained within +/- 1°C of the required setting, contact a repair company.
- II. Maintenance: Empty, clean and refill with fresh water as needed.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Thermometers**

I. Temperature Checks for NIST Thermometers or NIST Calibrated Thermometers

A. Procedure:

1. Check the thermometer only at the temperatures designated by the manufacturer.
2. If checking at 0°C, use an ice bath. If checking at 100°C, use a boiling water bath.

B. Frequency: Annually.

C. Results: Record the results in a log book. Computerized records will be backed up.

D. Course of action: If the temperature is not within +/- 2 degrees from the manufacturer's recommended reading, the NIST thermometer will not be used.

II. Temperature Checks for NIST Traceable Thermometers

A. Procedure:

1. Check the thermometer only at the temperatures designated by the manufacturer. This should be done simultaneously with your NIST or NIST calibrated thermometer.
2. Check the 0°C reading in an ice bath. Check the 100°C reading in a boiling water bath.

B. Frequency: Annually.

C. Results: Record the temperature reading for the thermometer in a log book. Computerized records will be backed up.

D. Course of action: If the temperature is not within +/- 2 degrees from the NIST or NIST calibrated thermometer, it will not be used

III. Maintenance: Maintain the original certification for the NIST or NIST calibrated thermometer.

**Forensic Biology/DNA Quality Assurance  
Equipment Function Checks and Maintenance  
Biohood Certification**

I. Checks and Certifications

A. Procedure:

1. Biohoods will be maintained by a company qualified to provide a certification of the biohoods' specifications.
2. All biohoods used for DNA will be certified before use in casework.
3. Certification should include, but is not limited to, a check of: the HEPA filter for leaks, the velocity of the air movement, and electrical safety.
4. The laboratory's annual internal audit will include a check of the UV illumination intensity by the Director of Quality Assurance or his representative. The intensity of the UV source will be a minimum of 200 mW.

B. Frequency: Certify the biohood and check the UV illumination annually.

C. Results: Record the results in a log book. Computerized records will be backed up.

D. Course of Action: If the certification or light check reveals parameters that do not meet specifications, contact the vendor or manufacturer for repairs and/or replacement parts.

II. Maintenance: Change light bulbs and HEPA filters when needed, or as indicated by the certification process.

## **Forensic Biology/DNA Quality Assurance Proficiency Testing**

- I. Proficiency testing is performed in accordance with Laboratory QA Manual, which includes the following:
  - Competency Tests
  - DNA Quality Assurance
  - Internal Proficiency Tests
  - Blind Proficiency Testing
  - External Proficiency Testing
  
- II. The results of proficiency test results will be checked and compared to the standards by the Quality Assurance manager as outlined in the Laboratory QA Manual.

## **Forensic Biology/DNA Quality Assurance Audits**

- I. All DNA laboratories will be internally audited once a year according to guidelines established in the Laboratory Quality Manual.
  
- II. In addition to the Laboratory's inspection program, external auditors will review the DNA section in the laboratory once every two years according to the ASCLD/LAB criteria and the FBI DNA Quality Assurance Audit Document.
  - A. A record of the audit report will be maintained in the laboratory.
  
  - B. A copy of the external audit report will be sent to the Quality Assurance Program Administrator with an action memo addressing issues identified by the auditor.

## **Forensic Biology/DNA Quality Assurance DNA Help Desk**

The DNA Help Desk assists analysts and answers questions that may arise about casework and the DNA Procedures Manual. The Help Desk consists of up to three individuals from the DNA section selected by Laboratory. Each of these individuals will serve a staggered three year term. These individuals are experienced DNA analysts selected for their level of casework experience and their technical knowledge of DNA procedures. The DNA Statewide Technical Leader has oversight of the Help Desk.

Purpose of the DNA Help Desk:

- To assist analysts with casework questions
- To assist analysts with questions concerning the DNA Procedures Manual
- To help disseminate information regarding DNA casework

## **Forensic Biology/DNA Quality Assurance Reports/Case Files**

- I. All case notes and supporting documents must be paginated.
- II. The case file should contain notations of the following, when applicable:
  - A. The packaging at the time of receipt, including if the package was sealed.
  - B. The description, a sketch, and/or a photograph of the evidence item that shows the size, shape, pattern and appearance of any stains. The location of the stains and where the stain material was removed.
  - C. The order the samples are extracted for DNA analysis must be clearly documented in the case notes. This must show the separate handling of the unknowns and their manipulation blank(s) from the standards and their manipulation blank(s).
  - D. Disposition of evidence items and/or remaining stain specimen, including repackaging and any sub-exhibits produced.
  - E. If assistance is received from an evidence technician for preparing blood standard cards, yield gels or slot blots, the technician must initial the worksheet picture or lumirad or image produced.
  - F. Multiple case numbers will appear on one worksheet whenever a procedure is performed on a group of samples. The worksheet must list all of the samples involved in the procedure. Photocopies of these worksheets are acceptable for the case file, but they must contain the original initials of the analyst or technician.
  - G. All testing performed and the results.
  - H. The initials of the technical reviewer and the date of the review.
- III. Report wording guidelines are found in Appendix I of the FB/DNA Procedures Manual.
- IV. For more efficient review, DNA case files will generally be organized as follows:  
  
Numbered Pages
  1. DNA Cover Sheet
  2. Correspondence
  3. Note-taking Worksheet

4. Yield Gel
5. Slot Blot
6. Amplification
7. Electropherograms (In the same order they are listed on the Summary Sheet)
8. Summary Sheets
9. Statistics
10. CODIS
11. Other Case Related Materials (Photocopies of biologist's report and notes, medical reports, etc.).

Non-numbered Pages:

1. Original DNA Report
2. Evidence Receipts

## **Forensic Biology/DNA Quality Assurance Reviews**

I. **Administrative Review:** All case files are required to have an administrative review. Prior to the submission of a case file for technical review, all cases must be reviewed by the author of the report for clerical accuracy, technical accuracy and completeness. Documentation of the administrative review will be the signature of the Forensic Scientist on the report.

II. **Technical Review:** All DNA case files must be technically reviewed by another qualified analyst to ensure technical accuracy. Biology case files will be technically reviewed according to the guidelines established in the Laboratory Quality Manual, QM-7.

If a technical reviewer has questions or concerns about information contained within the file, it should be brought to the attention of the Forensic Scientist for resolution. If a difference of opinion occurs between the Forensic Scientist and the technical reviewer regarding this concern or question, the issue should be handled as outlined in the Quality Manual, QM-7 for all biology cases. For DNA cases, the issue should be brought to the attention of the Statewide Technical Leader.

III. **Supervisory Review:** These are outlined in the Laboratory Directives and in the Laboratory Quality Manual. The supervisory review form may be used as a guide for the reviewer. It may be retained by the administrator as documentation of the review, but is not part of the case file.

IV. **Court Monitoring:** This is covered in the Laboratory Quality Manual under Administrative Reviews and Courtroom Testimony Reviews

## **Forensic Biology/DNA Quality Assurance DNA Supervisory File Review Procedure**

**Purpose:** To ensure that proper note taking and case file documentation procedures are followed.

**Procedure:** The reviewer must note the following:

### 1. General considerations

\_\_\_\_\_ Are all items in the file marked with the case number and additional information (such as the item number, analysts initials and date) where appropriate?

\_\_\_\_\_ Are all case note pages numbered?

\_\_\_\_\_ Are the total number of pages of notes included?

### 2. Chain of evidence

\_\_\_\_\_ Check the evidence receipt for accuracy and completeness.  
Has the chain of evidence been appropriately documented on the evidence receipt?  
Are all items of evidence tracked adequately?

\_\_\_\_\_ Are all supporting documents (e.g., locker receipts) in the file?

\_\_\_\_\_ Check the report for accuracy and completeness of chain.

\_\_\_\_\_ Cross check the report and the evidence receipt.

\_\_\_\_\_ Check the notes.  
Were additional exhibits generated and tracked appropriately?

### 3. Technical Documentation

\_\_\_\_\_ Check the case file cover sheet for completeness.  
Did the technical proofer initial and date the cover sheet?

\_\_\_\_\_ Check for case notes.  
These must include a description of what evidence was received and any additional exhibits generated.

## **Forensic Biology/DNA Quality Assurance Safety**

- I. The safety program is found in the Laboratory Safety Manual.

[Return to Protocol Index](#)

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