

# Unit Policy Manual

Forensic Biology

Archived Version

**SAN DIEGO POLICE DEPARTMENT**

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Authored and Approved by: S. Montpetit, DNA Technical Manager

Issuing Authority: F. Healy, Quality Assurance Manager

# Unit Policy Manual

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## Forensic Biology

### 1.0 QUALITY ASSURANCE

#### 1.1 UNIT DESCRIPTION

The Forensic Biology Unit is comprised of Criminalists, a DNA Technical Manager, and two Supervising Criminalists. The unit is generally staffed from 0700-1700hrs Monday through Friday.

The Forensic Biology Unit is divided into three distinct areas on the 6th floor of the SDPD headquarters building: an area dedicated to the screening of evidence; the main laboratory area which is used for the purposes of screening some items of evidence, the DNA extraction process, and PCR setup; and a separate area dedicated to the preparation and analysis of amplified DNA.

#### 1.2 UNIT FUNCTIONS

The Forensic Biology Unit aims to provide analysis on all reasonable requests from any investigative units of the San Diego Police Department using the most current technologies available.

The Forensic Biology Unit's primary duties are to analyze items of physical evidence to locate and identify biological material, and perform DNA analysis on that biological material.

The biological materials that the Unit can presumptively identify, or confirm the presence of, are blood, semen, saliva, and feces. DNA analysis may be performed on all of these materials as well as hair, bone, and other body fluids or tissues.

The DNA testing capabilities of the Forensic Biology Unit include both autosomal and Y-chromosome STR DNA testing, including as mini STRs. Mitochondrial DNA testing is not currently performed at the San Diego Police Department Crime Laboratory.

Additional duties performed by the Forensic Biology Unit include the validation and implementation of new technologies, courtroom testimony, and crime scene reconstruction.

# Unit Policy Manual

## Forensic Biology

### 1.3 PERSONNEL AND JOB DESCRIPTIONS

#### 1.3.1 SUPERVISING CRIMINALIST

The Supervising Criminalist of the Forensic Biology Unit will oversee the Forensic Biology Unit and analysts. The responsibilities will include assigning casework, interacting with attorneys and detectives to determine case priorities, reviewing the work product of all analysts, and monitoring the overall job performance of analysts.

Duties and responsibilities: (The duties of Supervising Criminalist and DNA Technical Manager may be assigned to a single person.)

- A. Establishes casework priorities based on negotiations and discussions with investigators and district attorneys.
- B. Assigns casework to analysts.
- C. Reviews reports and examines case notes.
- D. Reviews and evaluates the performance of subordinates.
- E. Works with the DNA Technical Manager to review and establish Unit specific policies and analytical procedures when appropriate.
- F. Interviews and hires new employees.
- G. Trains the new employees on department policies and procedures.
- H. Works with the DNA Technical Manager to coordinate analyst training.
- I. Keeps track of workload statistics.
- J. Prepares budget requests.
- K. Holds Unit meetings to disseminate department, laboratory, or unit specific information.
- L. Prepares reports as required by the Crime Laboratory Manager.

#### 1.3.2 DNA TECHNICAL MANAGER

The DNA Technical Manager will oversee the technical operation of the DNA Laboratory as outlined by the SWGDAM and the Quality Assurance Standards. The DNA Technical Manager will evaluate new

# Unit Policy Manual

## Forensic Biology

technologies, resolve technical problems, assess and design training programs, and ensure the unit adheres to all quality assurance guidelines. The DNA Technical Manager is authorized to shut down the DNA laboratory should a serious technical problem develop.

Duties and responsibilities:

- A. Responsible for the technical operation of the laboratory as outlined by SWGDAM.
- B. Reviewing, revising and approving any DNA-related technical policy or procedure prior to final approval by the QA Manager.
- C. Approving and overseeing training of new employees (when applicable) in DNA analytical procedures, including completion of training documentation.
- D. Overseeing, reviewing and approving DNA method validation, including completion of validation documentation, prior to final approval by QA Manager.
- E. Working with the QA Manager on any QA reports involving a DNA technical issue, and reviewing and signing off on those QA reports.
- F. Acting as a technical reference for the QA Manager.
- G. Performing some technical reviews of DNA case files.
- H. Acting as a mediator when necessary in the technical review of DNA casework in the Forensic Biology Unit.
- I. Providing technical consultation as needed to the members of the Forensic Biology Unit.
- J. Working with Supervising Criminalist and QA Manager to ensure compliance of the DNA analysts with QA and Unit policies and procedures.
- K. Reviewing the DNA proficiency tests completed by the Forensic Biology Unit.
- L. Conducting casework analysis.
- M. Prepares reports as required by the Crime Laboratory Manager.

### 1.3.3 CODIS ADMINISTRATOR

The CODIS Administrator is responsible for the security and integrity of the local CODIS network, the CODIS software, and the data within the local database. The CODIS Administrator ensures the data within the local CODIS database is in compliance with NDIS guidelines. The CODIS administrator is authorized to

# Unit Policy Manual

## Forensic Biology

terminate an analyst's or the laboratory's participation in CODIS until the reliability and security of the computer data can be assured if an issue with the data is identified. If the CODIS Administrator determines at any point that the CODIS network, software, or entered data has been compromised, the DNA Technical Manager will be immediately notified. The DNA Technical Manager and CODIS Administrator will determine the proper course of action to reinitiate participation in CODIS for the analyst(s) or laboratory..

Duties and responsibilities:

- A. Oversees the entering of DNA profiles from casework into the local database.
- B. Ensures that DNA profiles are searched against the local CODIS database.
- C. Uploads data to the California CODIS database.
- D. Downloads and searches DNA profiles from the CJIS WAN website.
- E. Performs upgrades to the CODIS software with the assistance of the CODIS Help Desk.
- F. Ensures that a backup is maintained for the information in the local database.
- G. Schedules and documents the yearly training of analysts in the operation of CODIS software.
- H. Disseminates CODIS information to the rest of the Forensic Biology Unit.

### 1.3.4 CRIMINALIST (I AND II)

The job descriptions for Criminalist I and II are similar except that Criminalist II duties may be more extensive and Criminalist II analysts are expected to work more independently. In addition to the normal educational requirements of the Criminalist position, DNA Criminalists must complete college-level classes in Biochemistry, Molecular Biology, Genetics, and Statistics and/or Population Biology.

Criminalist I and II's primary duties involve locating and identifying biological material on items of physical evidence and performing DNA analysis. Analysts are required to document all exams, tests, observations, results, summarize the findings in a written report, and if necessary, testify in courts of law.

Duties and responsibilities:

- A. Receives, inventories, screens, and preserves items of physical evidence.
- B. Locates and identifies biological material.

# Unit Policy Manual

## Forensic Biology

- C. Conducts DNA analysis to determine possible associations between the evidence and any reference samples provided.
- D. Writes reports summarizing the findings and provide expert testimony in court.
- E. Remains technically current and knowledgeable in appropriate analytical methods.
- F. Participates in the evaluation and validation of new technologies or methodologies.
- G. Participates in any special projects as assigned by the Supervising Criminalist or Laboratory Manager.
- H. Informs the Supervising Criminalist or DNA Technical Manager of problems that develop at any stage of a case.
- I. Uses the technical manual, unit policies manual and general quality assurance manual to deal with any procedural question or analytical method.

### 1.3.5 LABORATORY TECHNICIAN

The laboratory technician functions as a support position for the Forensic Biology Unit. Duties include ordering supplies, calibrating instruments, monitoring operating temperature of equipment, performing routine maintenance of equipment, and cleaning the unit on a weekly schedule. The laboratory technician maintains a database of all chemicals ordered by the Unit. If the position of Laboratory Technician is unfilled the duties and responsibilities will be shared among current employees. Certain functions described above may be performed by volunteers or interns but only under the direct supervision of the laboratory technician or other qualified analyst.

Duties and responsibilities:

- A. Checks the calibration of instruments such as pipettes, thermal cyclers, and balances.
- B. Orders chemicals and supplies for Forensic Biology
- C. Maintains an accurate inventory of all chemicals located in the Unit.
- D. Ensures that laboratory instruments, communal work areas, and floors are cleaned and decontaminated.
- E. Ensures that the operating temperatures of the Unit ovens, incubators, refrigerators, and freezers are monitored.
- F. Ensures that victim, suspect, and physiological, and reference mouth swab kits are prepared for departmental use or for the collection of evidence at area hospitals.

# Unit Policy Manual

## Forensic Biology

G. Performs other tasks as assigned by the Supervising Criminalist.

### 1.3.6 INTERN/VOLUNTEERS

The local universities serve as a source of interns and volunteers wishing to gain experience working in a crime laboratory. Interns and volunteers working in the Forensic Biology Unit will typically have earned or are working toward a four-year science degree.

Interns and volunteers will assist the Unit by performing certain clerical duties, cleaning the laboratory work areas and monitoring the operating temperature of equipment. These activities will always be performed under the direct supervision of the Laboratory Technician or Criminalist. The Laboratory Technician is ultimately responsible for making certain any duties completed by the interns and volunteers are performed correctly.

Depending on the experience level of an intern or volunteer they may also be involved in laboratory projects such as the validation of new technologies. This validation work will always be performed under the direct supervision of the DNA Technical Manager.

### 1.4 TECHNICAL LEAD CONTINGENCY PLAN

The Forensic Biology Unit of the San Diego Police Department currently employs multiple analysts who meet the requirements to be DNA Technical Manager. In the event of a vacancy in the DNA Technical Manager position the Crime Laboratory Manager will interview the candidates and appoint a person to fill the vacancy.

### 1.5 QUALITY ASSURANCE REVIEWS AND REPORTS

In general, Quality Assurance Reviews will be conducted when policy or method non-compliances are identified in administrative practices, evidence handling, casework, or in proficiency test situations. Additionally, a Quality Assurance Review will be conducted on any incident of Staff DNA Contamination or in any unexpected DNA results in controls that compromise the interpretation or results. If a non-compliance is found to exist, then a Quality Assurance Report will be issued. Additionally, a Quality Assurance Reports will be issued on any incident of Staff DNA Contamination or in any unexpected DNA results in controls that compromise the interpretation of the results. Please see policy 7.5 "Noncompliance review and corrective action protocol" in the General Quality Assurance Manual for further information.

### 1.6 PROTOCOL FOR UNEXPECTED RESULTS

# Unit Policy Manual

## Forensic Biology

Detectable DNA results from a reagent blank or amplification blank, unexplainable extraneous DNA results in a positive amplification control, or negative results from a positive control are considered unexpected results. Other types of unexpected results may exist.

Any unexpected results will be documented by the analyst conducting the analysis. The analyst will complete an "unexpected result summary" [*template located on the FB network H:\Worksheets\QA worksheets*] for inclusion in the "Unexpected Result Log". This log will be maintained by the DNA Technical Manager. The DNA Technical Manager and the Supervising Criminalist(s) of the Forensic Biology Unit will be notified of any unexpected results, review, and sign off on the summary. The Quality Assurance Manager will review all unexpected results.

The summary sheet completed by the analyst will contain the following information: The case number, analyst name and ID number, date of summary, date of result, and the definition and evaluation of the problem. The summary will also detail the resolution of problem, if any, as well as the conclusion/result/interpretation. If any protocol, policy, or reagents will be changed as a consequence of the unexpected result then these will be documented as well.

For a data set containing a control (reagent blank, positive amplification, negative amplification control, formamide blanks) with unexpected types to be relied upon, the unexpected results should not affect the interpretation (the detected types should be sample specific to the control). For any data set containing a control with unexpected detectable DNA types, the unexpected results normally will be disclosed in the report for the associated evidence samples.

### 1.7 FORENSIC BIOLOGY UNIT INCIDENT LOG

The purpose of the FB incident summaries are to monitor any incident that could indicate a problem with any lab equipment or instrument. The FB Unit Incident Log will monitor unusual events that occur during casework that:

1. Are corrected prior to the issuance of any reports for the affected cases.
2. Do not ultimately affect the reported results.
3. Do not require formal QA documentation, unless otherwise determined by the DNA Technical Manager or Quality Assurance Manager.

The unusual incidents will be documented by the analyst conducting the analysis. The analyst will complete an "Incident Summary" [*template located on the FB network H:\Worksheets\QA worksheets*] for inclusion in the "FB Incident Log". This log will be maintained electronically by the DNA Technical Manager. The DNA Technical Manager and the Supervising Criminalist(s) of the Forensic Biology Unit will be notified of any unusual incident, review, and sign off on the summary.

The summary sheet completed by the analyst will contain the following information: The case number(s), analyst name and ID number, date of summary, date of the incident, and the definition and evaluation of the problem, as well as its resolution. Supporting documentation, if available, will be provided with the summary.

### 1.8 PROFICIENCY TESTING



# Unit Policy Manual

## Forensic Biology

Please refer to the Laboratory Quality Assurance Manual for additional information on the proficiency testing Program.

Each DNA analyst in the Forensic Biology Unit must complete two proficiency tests in each calendar year with an interval between the tests of no less than four months and no more than eight months. Both of these proficiency tests will be external and at least one will involve DNA typing and/or body fluid identification. Analysts that perform Y-screen testing are considered DNA analysts for proficiency test purposes and will be tested twice a year using a team approach with a fully qualified DNA analyst. Analysts conducting only traditional screening testing will be proficiency tested once per calendar year. For DNA proficiency tests, the due date of the proficiency test (as listed by the test provider) will be the date by which these tests are tracked.

Proficiency tests will be worked in the same manner as casework and undergo both technical and administrative review. Under no circumstances should analysts compare proficiency test results prior to submitting them for technical review.

DNA analysts are required to perform at least one organic and one automated DNA extraction per year for the proficiency tests. Both methods may be used on a single proficiency test. GlobalFiler and Yfiler (for those qualified) must be run on each semiannual proficiency test. Yfiler testing must be performed on any samples with male DNA. If male DNA is detected in multiple fractions of the same sample (i.e., sperm and non-sperm fractions), Yfiler testing is required on only one of the fractions. MiniFiler must be run on proficiency test samples at least once each year.

Results from the DNA proficiency tests will be submitted to the test provider. When the results from the test provider are available, the DNA Technical Manager will compare the results obtained by each analyst and inform them of the outcome. Analysts will initial their proficiency test packets, acknowledging the outcome, and the test packets will be maintained by the Quality Assurance Manager.

### 1.9 FORENSIC BIOLOGY COMPUTER NETWORK SYSTEM

The Forensic Biology Unit employs an independent network system comprised of several servers and client computers to share electronic data among criminalists, link instrumentation, maintain a centralized storage of data, and provide a secure connection to the Combined DNA Index System (CODIS).

#### 1.9.1 ELECTRONIC DATA STORAGE

Electronic data is saved on a daily basis (Monday to Friday) on hard drives and tapes. The tapes are secured in a fire proof safe located in the Forensic Biology Server Room when not in use. Every quarter a selected number of tapes will be removed from the Forensic Biology Server room for submission and final disposition at an off-site location. Iron Mountain is the company currently used by the San Diego Police Department for off-site data storage. Contact SDPD Data Systems regarding a schedule pickup and/or retrieval of tapes with the following information: Customer # 106062 and description of tape.

# Unit Policy Manual

## Forensic Biology

### 1.10 LITERATURE REVIEW

Analysts within the Forensic Biology Unit will be provided copies of relevant literature (full articles, abstracts, or miscellaneous publications) periodically throughout the year. Analysts will document their review of the circulated literature by indicating they have viewed it on the attached routing slip. The circulated literature and signed routing slips will be maintained by the DNA Technical Manager.

### 1.11 QUALITY CONTROL AND CRITICAL REAGENTS

The following list contains the reagents deemed critical to the DNA testing process:

All reagents used in the organic extraction of DNA (excluding dithiothreitol (DTT))  
Qiagen DNA Investigator and QiaSymphony DNA Investigator Kits  
All Quantifiler qPCR Kits  
All Autosomal or Y-chromosome STR Typing Kits

Reagents/kits are deemed critical if the improper preparation or production of the reagent/kit could lead to an analyst failing to obtain a DNA type or obtaining the incorrect DNA type.

#### 1.11.1 SAMPLES AVAILABLE FOR QUALITY CONTROL TESTING

Only samples with known DNA profile results will be used to assess critical reagents. Extraction reagents will be quality tested using dried blood samples. DNA typing and quantification kits will be quality tested using previously extracted DNA samples.

#### 1.11.2 QUALITY CONTROL TESTING OF REAGENTS AND KITS

Before any critical reagent or kit can be used on casework samples it must be tested in a way that would reveal any problems with its composition and/or function. All quality control analysis will undergo both technical and administrative review. The genotypes of the positive controls, NIST SRM, and known samples must be in complete agreement with their previously known STR types. The negative control should yield no detectable DNA results. If casework analysis using the new reagents or kits is required before final completion of the technical and administrative reviews, approval must be obtained from the DNA Technical Manager prior to using the kits.

In the event that the QC data is unacceptable, a second run of the QC samples can be used to demonstrate the reliability of the reagent or kit. If the cause of any failure is deemed to be reagent or kit related, then the critical reagents tested will not be approved for casework and, if applicable, the vendor of the reagent will be contacted. In-house reagents will be re-prepared and quality tested before use.

# Unit Policy Manual

## Forensic Biology

The critical reagents/kits will be tested as follows:

Extraction Reagents: Two different known samples and a reagent blank will be carried through DNA extraction and typing. The preparation dates of the reagents and the results of the DNA testing will be documented on the Quality Control Worksheet for Critical Extraction Reagents.

Qiagen (EZ1 or QiaSymphony) DNA Investigator kits: Two different known samples and a reagent blank will be carried through DNA extraction and typing. The kit, reagent strip lot numbers, and the results of the DNA testing will be documented on the Quality Control Worksheet for DNA Investigator kits.

Quantifiler kits: The DNA standard tubes from the new lot of Quantifiler (Human or Duo) kits should be defrosted, pooled, and re-distributed into the DNA standard tubes. The new lot of kits should be used to quantify calibrators 1 and 2 from the old lot of kits as well as three replicates of calibrators made with the current lot of kits. Two samples with known genotypes should also be quantified using the new Quantifiler kit(s), the obtained quant values should be used to amplify those samples (and appropriate controls) using one of the validated typing kits. All samples should produce the known genotypes and the peak heights should generally be consistent with expectations based on the input amount of DNA. The target volume of the QC samples will be determined based on the quantification value obtained from the new Quantifiler Kit. Successful typing of the known samples will serve to demonstrate the reliability of the new kit lot(s).

GlobalFiler DNA typing kits: Two known samples will be typed along with the 007A positive control and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for GlobalFiler. (See also the NIST Testing policy below)

Yfiler DNA typing kits: New kit lots will be tested with two known samples, the 007 DNA positive amplification control, and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for Yfiler.

MiniFiler DNA typing kits: Two known samples will be typed along with the 007 DNA positive control and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for MiniFiler.

Identifiler Direct DNA typing kits: Two known samples will be typed along with the 9947A DNA positive control and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for Identifiler Direct.

All QC data will be stored electronically on the FB network.

### 1.12 NIST TESTING OF DNA PROCEDURES

# Unit Policy Manual

## Forensic Biology

Once a year, the DNA typing process will be checked against NIST or NIST-traceable standards. Previously extracted known DNA samples can be tested alongside NIST SRM samples, thus making them NIST traceable. If kits are quality controlled using these NIST-traceable samples, this check will serve as the yearly check against NIST standards.

### 1.13 PERFORMANCE CHECKS

Performance checks will be performed on the following equipment that has been identified as being critical to the analysis of samples in the Forensic Biology Unit. The performance of this equipment will be assessed at approximately the intervals indicated.

A performance check will be performed on any of the instruments listed below if any maintenance, repair, or service is conducted.

Equipment Item	Location	Performance Check Schedule (approximate)
Pipettes	Forensic Biology Main Lab and Amplification Room	Every six months
Balances	Forensic Biology Main Lab and Amplification Room	Every three months
BioRobot EZ1s Volume Test	Forensic Biology Main Lab	Every six months
BioRobot Universal	Forensic Biology Main Lab	Every six months
Hamilton Nimbus	Forensic Biology Main Lab	Every six months
Thermal cyclers	Amplification Room	Every six months
SDS 7500 Block Contamination Check All other calibrations	Amplification Room	Every six months Annually
Genetic Analyzers	Amplification Room	Annually
Corbett CAS-1200	Amplification Room	Annually

# Unit Policy Manual

## Forensic Biology

Pipettes – a performance check of a pipette consists of an evaluation as to the accuracy of the volume delivered against the selected volume. A passing result must be within 5% of the selected volume.

Balances – see section 6.4

EZ1 Volume Test – see section 6.5.3

BioRobot Universal – see section 6.7

Hamilton Nimbus – Pipetting precision and accuracy of the Nimbus is evaluated through replicate assessment of delivered volumes of liquid. The high volume (1mL) must be within 2% for accuracy and within 1.5% for precision. The low volume (10 $\mu$ L) must be within 9% for accuracy and within 6% for precision.

Thermal Cyclers – see section 6.8.8

SDS 7500 – see section 6.8

Genetic Analyzers - the QC analysis of critical reagents will serve as the performance checks of the Genetic Analyzers. The QC runs performed for the various testing kits employed by the lab will be rotated through the various Genetic Analyzers throughout the course of the year. Performance checks separate from QC runs may also be done using a minimum of the amplification positive, amplification negative and allelic ladder.

Corbett CAS-1200 – the performance check of the CAS-1200 will consist of an evaluation of the instruments pipetting against manual pipetting of the same samples. This will be accomplished based on the CAS-1200 pipetting of a known set of samples for analysis on the genetic analyzer. The results of the CAS-1200 pipetted samples will be compared against the same samples prepared manually. Total detected peak heights of both sets of samples will be used to measure the performance of the CAS-1200. The difference between the total peak heights both sets of samples should be less than 10%.

### 1.14 POLICY FOR MONITORING INCUBATORS, REFRIGERATORS, AND FREEZERS

The incubators, refrigerators, and freezers in the Forensic Biology Unit will be monitored approximately every week. The operating temperatures should be recorded on a worksheet attached to the equipment. Refrigerators will be expected to operate at a temperature between 1 to 13°C and freezers at a temperature colder than -10°C. Consistent deviations from the expected temperature indicate that maintenance or repair is likely required and the Supervising Criminalist of the unit will be notified.

#### 1.14.1 MICROSCOPES

# Unit Policy Manual

## Forensic Biology

The microscopes in the Forensic Biology Unit will be maintained according to policies outlined in the laboratory Quality Systems Manual.

### 1.15 QUALITY ASSURANCE LOGS FOR THE FORENSIC BIOLOGY UNIT

This list below indicates all the logs maintained by the Forensic Biology Unit:

Log Name	Location
Kit Receipt Log	Main FB
Reagent Preparation Logs	Main FB
Supply Order Log	Main FB
DNA Extraction	Electronic
Qiagen Robot Reagent QC	Electronic
Identifiler Plus QC	Electronic
Y-STR QC	Electronic
MiniFiler QC	Electronic
Quantifiler / Quantifiler Duo QC	Electronic
Unexpected Results	Electronic
pH Meter Calibration	Main FB
Balance Calibration Tests	Electronic
Pipette Calibration	Electronic
9700 Thermal Cycler Calibration & Uniformity	Electronic
Ovens & Fridges Temperature Log	Electronic
Qiagen EZ1 Volume Test	Electronic

# Unit Policy Manual

## Forensic Biology

### 2.0 CASE PACKETS AND REPORTS

#### 2.1 REPORT FORMAT

The Biology Unit will use the standardized report format located in the Quality Systems Manual. Case number, incident number, and date of occurrence will appear in the report header whenever possible. In addition to the items listed in the Report Standard Format section of the Quality Assurance Manual, the date of the report will be included in the initial header and on each subsequent page for multi-page reports. In general, items described in the report will be limited to those examined or tested. Where available, CII numbers will be included in the report.

##### 2.1.1 BACKGROUND SECTION

Any references to external data used to render conclusions in the current report must be referenced in the background section, or in the conclusion section if citing information relating to a database hit. The use of the background section to provide additional details that may be relevant to the reader(s) of the report is optional.

##### 2.1.2 CASE-TO-CASE NOTIFICATIONS

When providing information to investigators regarding links between evidence in different cases, tabular case-to-case hit notifications will be issued detailing the various cases linked through DNA evidence. These notifications must be technically and administratively reviewed prior to issuance.

Case-to-case notifications will be required when an unsolved SDPD case has been linked through the database to any unsolved SDPD case or any case from an outside agency. In general, case-to-case notifications will be issued for any newly solved cases linked to previously solved cases with the same suspect (no notifications are necessary for the previous case(s)).

If a newly analyzed unsolved case is linked to a previously solved case or cases, a case-to-case notification will be issued for the new case. The determination of whether additional notifications will be issued for the older cases will depend on case type, the statute of limitations on the old cases, and/or the time interval between cases.

##### 2.1.3 SCREENING REPORTS

Screening results can be summarized either through the inclusion of a table or through written explanations of the results.

#### 2.2 CASE PACKETS

The final packet assembled by the analyst will contain the original of the final signed report, complete analytical record (or a reference as to where the data is located), and other important administrative documents (e.g., work requests) for scanning and storage by the Clerical Unit. The final packet is subject to technical and administrative reviews before the packet is filed.

# Unit Policy Manual

## Forensic Biology

Numbering of administrative documents as part of the analytical record will be at the analyst's discretion. All additional documents in the case packet (e.g., administrative documents) will be denoted as administrative document (or Admin. Docs.) and include the case number on the first bound page or on all unbound pages. Administrative documents should be binder clipped or paper clipped together.

No loose items such as sticky notes or photos will be included in the packet. Small items must be mounted on 8 1/2" x 11" sheet paper.

If any information is referenced within the analytical record (e.g., a reference sample worked as part of a different case), a pink sheet indicating that the report relies on information contained within separate case packets. This sheet will serve to notify clerical that additional material is required in the event of a discovery request.

### 2.2.1 ANALYTICAL RECORD

In general, the analytical record will contain the following:

- Chain of custody information
- Documentation of relevant communications
- Evidence inventory and analysis notes, including any serology tests results
- DNA extraction, quantification, amplification, and capillary electrophoresis notes
- DNA results (electropherograms)
- Interpretation information/worksheets
- Match probability estimates/calculations
- CODIS worksheets/match estimator printouts for evidentiary samples
- Disposition information for work product

### 2.2.2 CASEWORK BATCHING

To streamline the administrative burden (case, packet assembly, technical review, and administrative review) of case processing large numbers of cases together, analysts may group the analytical records for multiple cases together into a single analytical record. Each case in the casework batch should receive its own individual report.

Alternatively, the analytical worksheets common to multiple cases (i.e., extraction through GMID-X) can be technically and administratively reviewed separate from the complete analytical record. The technical and administrative reviews shall be documented in the complete case packet, and the analytical batch only referenced. These analytical batches must be stored on the FB network such that they are available for review when the complete case packets are reviewed. Please see section 2.6.1 for additional information on technical review.

### 2.2.3 NOTETAKING

The chain of custody worksheet requires, at minimum, the case identifier, a unique identifier of the item(s) received, and the date and locations obtained/returned. The Chain of Custody Worksheet together with the body of the notes must uniquely describe the packaging, condition of the item, and complete contents of the item received.



# Unit Policy Manual

## Forensic Biology

Analysts should avoid using the names of victims of sexual assault or child abuse cases on worksheets containing information from multiple cases to reduce the risk of the information being revealed through unrelated cases.

The analyst will take notes with the expectation that they, a technical reviewer, or perhaps an outside expert, upon review (even years later) will be able to reconstruct what was done in terms of examinations conducted, testing completed, and any sampling.

Photographs will be labeled with a descriptor unless it is completely evident what the photograph represents. Photographs should ideally include scale.

Digital photographs taken in the Forensic Biology Unit will be archived so that they can be easily retrieved, if needed, for discovery. At a minimum, the electronic files of the photographs should be saved with the barcode of the item in the file name in a folder labeled with the case number.

### 2.3 INTERPRETATION OF CONTROLS

#### 2.3.1 SUBSTRATE CONTROLS

For any analysis, a result obtained from a questioned stain is interpreted as being representative of the stain when the result from the substrate control is negative.

Results from questioned stains for which the substrate control yields a positive result need to be interpreted with caution. A result from a questioned stain may not be representative of the stain when the substrate control also yields a positive result. In this circumstance, the results obtained from the questioned stain need to be considered along with the results obtained from the substrate control.

Some considerations for evaluating the substrate control include:

- A. The collection of the substrate control. (e.g. Was the control collected from a proven negative area?)
- B. Results from presumptive test versus a quantitative test. Was the substrate control originally evaluated as being negative by a presumptive test, which is less specific than the confirmatory test?
- C. Did the substrate control give a low level DNA result versus a considerably higher level DNA result from the questioned stain?
- D. The source of the substrate. Is there a stain on the substrate control, which was not identified presumptively, which could be giving the result? It may be necessary to examine the substrate for other body fluids.

#### 2.3.2 DNA CONTROLS:

# Unit Policy Manual

## Forensic Biology

Please refer to the Autosomal STR Interpretation Guidelines (or Y-STR Interpretation Guidelines section) section of the Forensic Biology Technical Manual for the policies regarding the interpretation of the *Reagent Blanks*, *Positive Amplification Controls*, and *Negative Amplification Controls*.

### 2.4 CASEWORK REVIEW

All casework performed by the Forensic Biology Unit is subject to both technical and administrative reviews. Refer to the casework review policy in the Quality Systems Manual.

#### 2.4.1 TECHNICAL REVIEW

Each case packet prepared by an analyst in the Forensic Biology Unit is subject to technical review by a second qualified analyst. Technical review will rotate between all qualified analysts in the Forensic Biology Unit to ensure that the process remains objective.

The purpose of the technical review is to check the accuracy of the final report and ensure that established Forensic Biology protocols are being applied to case analysis. The final report should adhere to the established laboratory format (see the Quality Systems Manual for more information) and the conclusions in the report must be supported by the data in the analytical record (case notes, sketches, photos, etc.).

The analytical record serves as the basis for all conclusions set forth in the final report and will be evaluated in the technical review process. The analytical record will be examined by the technical reviewer to ensure that it is complete and current Forensic Biology Unit procedures (including interpretation guidelines) and policies for the examination and testing of biological evidence were followed. Any material changes to the analytical record that affect the interpretation of any result, or the statistics applied to a DNA association, made as a result of the technical review, will be kept as part of the analytical record. Analysts must note that the changes were as a result of the technical review process.

The goal is to have the initial round of technical review completed with a two calendar week period. Any final review after a file is re-submitted to the technical reviewer should be completed within a few days of the re-submission. The date of the completion of the technical review will be documented in the report and in the analytical record. The completion dates of the report and analytical record can be different. It is the responsibility of the analyst to let the technical reviewer know of any rush status on a case given for review. It is the responsibility of the technical reviewer to communicate with the analyst if anything (vacation, training, etc) will delay the completion of the review.

Unresolved differences between the case analyst and the reviewing analyst can be addressed with the DNA Technical Manager.

# Unit Policy Manual

## Forensic Biology

The following list is meant to guide analysts in providing a complete technical review of case packets. The list is not meant to be all inclusive and a case needs to be evaluated in its entirety to make certain that a reasonable, competent, and complete analysis was performed.

<b>Analytical Record</b>
All additional materials in case file labeled with "Admin Document" DNA request has supervisor and/or analyst initials Notation from supervisor or communication with detective documented for requested samples that were not tested All other requested evidence and reference samples tested Case file has pink case reference sheet and/or green stat sheet, if applicable Date, initials, page numbers, and case # / incident # on each page Corrections are lined out, dated, and initialed Chain of custody exists for all evidence during analysis in Forensic Biology If applicable, permission to consume evidence obtained and/or communicated All important communications concerning the case appear recorded
<b>Evidence Sampling</b>
There is an adequate description of the evidence packaging There is an adequate written or pictorial representation for evidence examined There is an adequate written or pictorial representation for samples collected If applicable, photographs (if affixed to page) have proper identifying information Proper methods were used to locate biological material Proper methods were used to identify biological fluids Appropriate controls were used for alternate light source and presumptive tests Substrate controls collected and analyzed where appropriate Documentation present that evidence was repackaged and sealed Sub-item designations added to packaging and comments section in FileOnQ If applicable, "SART Kit Tested" field is updated in FileOnQ
<b>Analytical Notes</b>
Reviewed (TR/AR) Analytical Batch review page present, if applicable Analytical notes not part of analytical batch contain necessary documentation If applicable, QA Incident Summary documentation present in notes or in Analytical Batch Suitable amounts of DNA amplified for evidence and controls for the STR system Positive Control results acceptable Amplification Blank (TE) results acceptable All associated Reagent Blanks have acceptable results
<b>Electropherograms</b>
Appropriate analysis method, panel, and size standard used for all samples Electropherograms present for all relevant samples If altered injection times used, controls were injected appropriately Multiple injections performed where necessary If multiple injections performed, results generally consistent/expected Artifact and allele changes labels appropriately and sufficiently

# Unit Policy Manual

## Forensic Biology

<p>Loci with common microvariant alleles (X.1 or X.3), have no stutter peaks or alleles above detection threshold that did not fully resolve from neighboring peaks</p> <p>Heterozygous peaks in apparent single source samples appear to be balanced</p>
<p><b>Interpretation</b></p> <p>Assumptions regarding number of contributors are documented</p> <p>Conclusions based on visual comparison are documented</p> <p>STRmix run for all appropriate samples</p> <p>STRmix input files imported correctly for evidence samples</p> <p>If applicable, MCMC conditioned on appropriate references and the text file was imported appropriately</p> <p>MCMC results appear to have appropriate genotypes, weights, and mixture proportions</p> <p>If applicable, MCMC run with appropriate informed priors</p> <p>STRmix database search results evaluated for possible staff matches</p> <p>Comparisons were made to all relevant reference samples and the input files were correct</p> <p>LRs do not have large disparities in results at any loci (i.e., LR = 0 at only a single locus)</p> <p>STRmix diagnostics within reasonable range of values</p> <p>For Y-STRs, designation of major and minor contributors is appropriate</p> <p>For Y-STRs, appropriate comparisons were performed and conclusions drawn</p> <p>For Y-STRs, appropriate statistical assessment of evidence performed</p>
<p><b>CODIS</b></p> <p>CODIS sheets present for applicable evidence</p> <p>If applicable, MME is calculated appropriately for eligibility for SDIS/NDIS</p> <p>CODIS sheets present for applicable suspects</p> <p>All CODIS sheets have appropriate case #, item #, contributor %, specimen category, and offense information</p> <p>All CODIS profiles have correct specimen category and DNA types</p>
<p><b>Report</b></p> <p>Header is correct on first page And correct on additional pages</p> <p>If applicable, Supplemental Report info and Background are appropriate</p> <p>Evidence examined contains correct barcode and appropriate description of items</p> <p>Evidence examined contains results of presumptive/confirmatory testing</p> <p>DNA typing kit(s) listed in Evidence Examined or in Conclusions</p> <p>Conclusions have appropriate barcode and description (and fraction for diffs)</p> <p>Conclusion lists number of contributors and mixture proportions, if appropriate</p> <p>Conclusions include appropriate verbal wording for LR for each contributor</p> <p>Reported LR for inclusions use lowest HPD for all four populations</p> <p>Reciprocal of point LR reported for exclusions with limited to strong support</p> <p>Point LR reported for inclusions with no effective sample size, and inclusions with greater than <math>10^6</math> difference between LR and HPD</p> <p>Disposition of evidence contains appropriate information</p> <p>Signature block is correct</p> <p>Analytical batch(s) listed in report</p> <p>If applicable, Quality Assurance Incident documented appropriately in report</p>

# Unit Policy Manual

## Forensic Biology

### ANALYTICAL BATCH REVIEW

The review of an analytical batch (see section 2.2.2) is designed to streamline the reviewed process by not requiring duplication of data review. The following list is meant to guide analysts in providing a complete technical review of the analytical material in a batch.

Packet	
<input type="checkbox"/>	Date on each page
<input type="checkbox"/>	Initials on each page
<input type="checkbox"/>	Page Numbers on each page
<input type="checkbox"/>	Analytical batch name on each page
<input type="checkbox"/>	Corrections are lined out, dated, and initialed
Extraction Worksheets	
<input type="checkbox"/>	Appropriate volume of Digest Buffer/ Prot. K (and DTT)
<input type="checkbox"/>	Correct Preparation date for Digest Buffer/ Prot. K (and DTT)
<input type="checkbox"/>	Incubation time $\geq$ required minimum
<input type="checkbox"/>	Appropriate elution volume
<input type="checkbox"/>	TE or dH <sub>2</sub> O Elution box checked
<input type="checkbox"/>	EZ1 instrument indicated for each sample
<input type="checkbox"/>	Correct Reagent Strip Lot #
<input type="checkbox"/>	Reagent blanks included
Quantitation Worksheets	
<input type="checkbox"/>	Correct Quantifiler (Human or Duo) lot #
<input type="checkbox"/>	7500 Instrument listed
<input type="checkbox"/>	Setup listed
<input type="checkbox"/>	Master Mix Preparation was accurately calculated
<input type="checkbox"/>	Calibrators are within appropriate range
<input type="checkbox"/>	TE Amp Blank is <i>undet</i> and IPC is green
<input type="checkbox"/>	All reagent blanks are <i>undet</i> and IPC is green
Amplification Worksheets	
<input type="checkbox"/>	Correct Amplification Kit (GlobalFiler, MiniFiler, Yfiler) lot #
<input type="checkbox"/>	Correct TE Prep Date
<input type="checkbox"/>	9700 Instrument listed
<input type="checkbox"/>	Setup listed
<input type="checkbox"/>	Master Mix Preparation was accurately calculated
<input type="checkbox"/>	Correct volume for Positive Control (9947A or 007) added
<input type="checkbox"/>	Correct volume for TE Amp Blank added
<input type="checkbox"/>	If samples combined, corresponding RBs are combined
<input type="checkbox"/>	Correct volume for Reagent Blanks added

# Unit Policy Manual

## Forensic Biology

<input type="checkbox"/>	Dilutions (and/or) concentration procedures listed below table
<input type="checkbox"/>	10X TE added box checked (if needed)
3500 Sample Setup Worksheets	
<input type="checkbox"/>	Formamide Blank and Formamide ISS included
<input type="checkbox"/>	Ladders included
<input type="checkbox"/>	Positive and negative control included
<input type="checkbox"/>	Reagent blanks included
<input type="checkbox"/>	3500 Instrument listed
<input type="checkbox"/>	Sample setup listed
<input type="checkbox"/>	Correct Reagent Lot #s (Formamide, ISS, Ladder)
GeneMapper Analysis (electronically viewed)	
<input type="checkbox"/>	Sample page included in packet
<input type="checkbox"/>	At least 1 evidence and 1 reference ladder passed
<input type="checkbox"/>	All GS600 – Appropriate peak heights and resolution
<input type="checkbox"/>	Positive Control passed
<input type="checkbox"/>	TE Amp Blank (No detected DNA types)
<input type="checkbox"/>	All Reagent Blanks (No detected DNA types)

### TECHNICAL REVIEW EXPECTATIONS

The following serves as a further clarification of the expectations with regards to the technical review process should differences of opinion arise:

1. The Analyst and Technical Reviewer will meet *in person* and have a verbal conversation in an effort to sort out any differences of opinion. Each should have the opportunity to professionally explain their point of view.
2. Assuming the analyst and technical reviewer cannot come to an agreement; the input of a DNA Technical Manager will be sought.
3. Should the issue raised in technical review have policy implications, the Technical Manager may need to consider a policy change. In this case, the Technical Manager will work with the Supervising Criminalist(s) and/or the Quality Assurance Manager to determine the best course of action.
4. Assuming an agreement between the Analyst, the Technical Reviewer, and Technical Manager cannot be achieved, the Quality Assurance Manager will be asked to assist the three parties in coming to a resolution for the technical issue.
5. Should the technical review issue still remain unresolved the Supervising Criminalist for Forensic Biology will be informed of the impasse.
6. Analysts involved in the discussion may elect to have further discussions with the Laboratory Director concerning the technical issue.

### 2.4.2 ADMINISTRATIVE REVIEW

# Unit Policy Manual

## Forensic Biology

Each case packet prepared by an analyst is subject to administrative review. This review is normally conducted by the Supervising Criminalist; however, in the event a Supervising Criminalist is unavailable the administrative review may be performed by another analyst within the section.

The Quality Assurance Manual details the basic elements of the administrative review process. In addition to those criteria enumerated in the Quality Assurance Manual, the following list is meant as a guide to providing a complete administrative review of case packets. The list is not meant to be all inclusive and a case needs to be evaluated in its entirety to make certain that the report and analytical record are complete.

Notes
The chain of custody during testing in the laboratory is documented?
Worksheets are complete?
Report
Conclusions in the report are supported by the data?
Statistical frequencies provided for DNA profiles when appropriate?
Evidence disposition is summarized?
General
Report and case notes have had a technical review by a qualified analyst?
Analyst has corrected or otherwise resolved all concerns raised by the analyst performing the technical review?
Statistics worksheet summarizing work performed on case (if required)?
CODIS worksheets for appropriate samples?

### 2.5 INTERNALLY PRODUCED DNA REQUESTS

#### Introduction:

If an analyst performs initial analysis on casework in the Forensic Biology Unit, where there are positive findings for which they will not be performing the subsequent DNA analysis, they will submit a DNA request to the Supervising Criminalist.

If subsequent DNA testing is being requested by an analyst, the screening report will indicate the submission of a DNA request, and the Supervising Criminalist should be contacted concerning the status of the request.

#### Guidelines for Request Submission:

If no history is given or multiple suspects may be involved, all potentially probative samples must be forwarded for DNA testing in order to maximize the potential of obtaining DNA profiles from all offending parties.

A DNA analyst may request further work on unanalyzed items of evidence after obtaining the initial round of DNA testing results. Any additional work should be coordinated through the Supervising Criminalist.

# Unit Policy Manual

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## Forensic Biology

DNA analysis of a case is often assisted by the inclusion of the original screening request, microscopy worksheet pages, or Y-screen quantification results. The number of epithelial cells may also be relevant in certain evidence and scenarios and should not be overlooked when providing results.

DNA analysts require additional information such as case scenario, consumption issues, reference standard availability, presence of consensual partners/multiple assailants, and the existence of other evidence that should, when available, be included on internally produced DNA requests. If only clothing samples are listed in a DNA request a summary of the screening results from the SART kit should be included in the request (if available). The DNA request form will include information on how much of the submitted sample(s) remains should additional testing be required. The DNA request will document what additional evidence is available in the case.

If a large cutting is necessary to obtain DNA results, multiple cuttings can be placed in separate tubes or the entire stain can be removed and impounded within a freezer packet.

If the case involves a crime against a person, especially homicide, sex crimes, or child abuse cases, screening analysts will consult with the Supervising Criminalist, or Deputy District Attorney, when consumption of the evidence may be required for DNA analysis (see the Consumption of Evidence Policy in section 3.2 for additional information). The goal is to determine if any impediments or issues related to consuming the evidence can be worked out prior to the DNA analysis. This information should be documented in a communication log and added to the DNA request.

When liquid blood is being used as a reference sample, between 10-15uL of liquid blood should be submitted for DNA testing. A reference bloodstain card must be prepared in this circumstance (suggested sample size = 1mL). This newly created item must be given a barcode through the File-on-Q system and its disposition documented in the report.



# Unit Policy Manual

## Forensic Biology

### 3.0 EVIDENCE POLICIES

#### 3.1 CASE SUBMISSIONS

The Forensic Biology Unit accepts case submissions from all investigative units with the Department. Case submissions are handled as described in the Quality Assurance Manual.

Department Order 13-30 requires a consent form when collecting DNA from a subject who is not being arrested for a felony, is not a 4th waiver subject, or who is not a victim of a sexual assault. The consent form is used to inform the subject of his or her right to refuse to have a sample collected, and will inform the subject that the sample, if collected, may be entered into a database for comparison with other profiles. Any reference DNA sample requested must have a form submitted in order to have the sample analyzed, unless it has been collected from a felony arrestee, court order, 4th waiver subject, or sexual assault victim. If no form was collected, permission from the Assistant Chief in the requestor's chain of command will be required to proceed with analysis. The consent form or documented Assistant Chief approval will be maintained as an administrative document.

##### 3.1.1 CASEWORK STATISTICS

Casework statistics are recorded for each case that is analyzed and are tallied in monthly statistics for the Unit. Supervisors report case statistics for the unit to the Laboratory Manager.

#### 3.2 CONSUMPTION OF EVIDENCE

General QA Policy:

When possible, sample(s) will not be consumed in testing so that a sufficient quantity is retained for reanalysis. If the entire sample(s) must be consumed during analysis, the assigned DDA will be consulted whenever possible. If there is an objection to the SDPD lab consuming the evidence, alternative arrangements will be made for the analysis of the sample(s) in question.

In homicide, sex crimes, and child abuse cases where no suspect is associated with the case, the analyst will consult with the Supervising Criminalist prior to consuming the sample. These consultations/communications will be documented in the case notes. The case investigator will be contacted to inform them of the need to consume the evidence.

In all cases, with or without a suspect and/or no DDA assigned to the case, a notification will be sent to the detective informing him or her of the decision to consume the sample(s). Three business days will be given to the detective to provide an opportunity to respond. The analyst will proceed with evidence consumption in the absence of a response.

# Unit Policy Manual

## Forensic Biology

### Discussion:

If the decision of an analyst is that consuming an item of evidence provides the best chance of obtaining a DNA result and there is an issue with the laboratory consuming the evidence, an analyst may agree to initially consuming half of the sample (predicated on the subsequent analysis of the remaining portion of the evidence if needed) or may suggest that an independent laboratory be selected to perform the analysis.

For cases where there is no DDA assigned, the investigators will be informed of the intent to consume the evidence through an email. For cases without suspects a form email has been created for the purposes of informing investigators about the consumption of any evidence. The requirement of the email allows for documented communication.

### 3.3 UNIT POLICIES FOR EVIDENCE HANDLING

In an attempt to maintain a high level of quality in all aspects of the analyses performed by the Forensic Biology Unit, the following policies and practices will be adhered to wherever possible:

#### 3.3.1 GENERAL:

See the laboratory's Quality Assurance Manual for general evidence handling requirements.

- Separate areas are designated within the Unit for DNA extraction, PCR setup, and for the analysis of amplified DNA. Procedures will be performed in the appropriate area for that analysis.
- Prior to analysis, the work area must be cleaned with a 10-20% bleach solution. The work area will be covered with clean paper or bench mats. The contents of items must be inventoried and examined on a fresh piece of paper. This paper can be used to wrap the item after examination, and should be marked with the item's identifying information.
- All items examined must be marked with a minimum of the barcode and analyst initials. If circumstances prevent or preclude marking the item directly only their proximal containers will be marked. Any analysts performing verifications need only mark their initials on an item.
- When evidence to be analyzed is retrieved from the Property Room or Laboratory Vault the condition of packaging will be noted (i.e., damaged, unsealed, etc). When opening an item, care must be taken to avoid compromising any information written on the packaging. If possible the original seal will not be broken. If the contents of the packaging cannot be accessed without breaking the seal, then a notation will be made in the case notes.
- An analyst will wear appropriate safety gear and disposable gloves when handling evidence within the unit.

# Unit Policy Manual

## Forensic Biology

- Slides or other derivative evidence generated as a result of analysis are generally given a new barcode in File-on-Q system, a notation as to final disposition will be made in the notes.
- Digital images will be taken of most evidence and color laser prints of these images will appear in the case notes. An archive of all digital images taken by Forensic Biology analysts is maintained on the FB network.
- After analysis, items will be returned to the original packaging if possible. The package must be sealed with evidence tape and the analyst must initial and date the seal. The barcode number must be on the outer packaging.
- For any procedure performed in the Forensic Biology Unit only one tube or evidence item should be open at a time during any sampling and reagent/DNA addition. Use of any robotics system for sample setup is an exception to this policy.
- Within the Forensic Biology Unit there are separate refrigerators and freezers dedicated to the storage of either reagents or evidence. Evidence and reagents should be stored separately in their designated locations while in the Forensic Biology Unit.
- If an evidence item is swabbed for DNA analysis, a notation will be made within the File-on-Q evidence tracking database regarding any sub-designations assigned, or additional associated barcodes created.

### 3.3.2 CASE APPROACH

- Communication between units is important in determining the sequence of examination. A unit able to individualize evidence has priority over a unit, which can only determine class characteristics. Destructive examinations will be carried out as a last resort.
- The danger of cross-contamination must be recognized and steps taken to prevent it. Evidence collected from the suspect must not be analyzed or extracted at the same time as evidence collected from the victim. The DNA extraction of evidence and reference standards must be separated by time and/or place.
- Cigarette butts will go straight to DNA analysis prior to any attempt at latent print development.
- It must not be assumed that swabs from the same area packaged separately are equivalent. If body cavity swabs from a sexual assault kit are submitted in multiple tubes, analysts are obligated to test swabs from both tubes.
- In general, if a single swab is collected in a Child Abuse case, the swab will be directly analyzed for DNA in an attempt to preserve as much DNA on the sample as possible. Any decision to initially consume less than a full swab for non-DNA testing should be discussed with a Supervising Criminalist. That communication must be documented in the case notes.

# Unit Policy Manual

## Forensic Biology

### 3.3.3 DNA EXTRACTION:

- When sampling for DNA analysis a clean cutting surface should be used for each piece of evidence. Clean scissors or scalpel blades thoroughly with bleach, soap and water, alcohol, or by flaming after cutting each evidence sample. Alternatively, use a fresh scalpel blade with each evidence sample.
- All reagents and solutions will be sterilized where possible. Sterile water and sterile plastics or glassware will be used for solutions that cannot be autoclaved.
- Reagents will be stored as small aliquots to minimize the effects of any contamination events. The lot(s) of reagents used for each set of analyses will be recorded so that if contamination occurs, it can be traced more readily.
- Sterile disposable aerosol resistant pipette tips and sterile tubes will be used for any processes involved in DNA analysis. Pipette tips will be changed between adding and/or removing liquid from evidence samples.
- It is advisable to briefly centrifuge all tubes before opening to remove liquid from the caps.
- The number of samples processed at one time should be limited to a manageable number. This precaution will reduce the risk of a sample mix-up and the potential for sample to sample contamination. The manageable number of samples will vary depending on the experience and comfort level of an analyst.
- The DNA extraction/purification of questioned samples will be performed separately from the DNA extraction/purification of reference samples from the same case. This precaution will help to prevent potential cross-contamination between reference and evidence samples. In addition, it is advisable that DNA extraction/purification of samples, where high amounts of DNA are expected, (references or large bloodstains) be performed separately from samples where low amounts of DNA are expected (single hairs or very small stains) to minimize the potential for sample to sample contamination.
- Only unamplified DNA samples will be stored in the refrigerators and freezers in the extraction area. No reagents used in the post amplification processes will be stored in any refrigerator or freezer in the DNA Extraction Area.
- At least one reagent blank will be employed for each set of DNA extractions/purifications to check for possible contamination of the DNA extraction reagents or DNA Extraction Area. A reagent blank is subjected to all of the reagents and manipulations of the DNA analysis process, but contains no sample. The reagent blank(s) should be treated in the same manner as the most manipulated evidence sample associated with it. The reagent blank will be carried through amplification and analysis with its associated samples. It is advisable to employ multiple reagent blanks in a batch extraction to accommodate the combining of samples, or the use of multiple DNA testing kits if any samples are

# Unit Policy Manual

## Forensic Biology

consumed during a first analysis.

- Performing the initial water wash and microscopic examination will be optional for samples that have previous serological, or Y-screen examinations performed. For reporting purposes, the previous analysts' results may be used to document the presence specific cell types or body fluids.

### 3.3.4 QUANTIFICATION

- The Quantifiler Duo quantification kit must be used on evidentiary samples derived from sexual assault cases. Other cases may benefit from the additional information this kit provides and the use of it on cases other than sexual assault cases will be at the discretion of the analyst.

### 3.3.5 PCR SETUP:

- Use dedicated pipettes for adding kit reagents and DNA to the PCR tubes.
- Add DNA to the PCR tube last. This practice reduces the possibility of inadvertent transfer of DNA between samples and kit reagents. Only one sample tube should be open at a time during DNA addition.
- The negative amplification control (no DNA) allows for the detection of contamination during PCR setup.
- Avoid touching the inside surface of the tube caps.
- Change pipette tips after the addition of each DNA sample.
- Store the amplification kits in the designated reagent refrigerator. Do not store them near extracted DNA.

### 3.3.6 POST AMPLIFICATION ROOM:

- Only disposable lab coats are to be used in the Amplified DNA Area.
- The Amplified DNA Area is dedicated to amplified DNA and should not be used for pre-amplification purposes.
- All surfaces in the Amplification Area should be cleaned with 10-20% bleach solution before and after setting up samples for analysis. The floor of the Amplification Area will be mopped with a dilute bleach solution every two weeks.
- Caution should be used when handling PCR products to minimize dispersing of the product in the Amplification Area.

# Unit Policy Manual

## Forensic Biology

- Always remove the gloves and lab coats before exiting the Amplification Area.
- Change gloves whenever contamination with PCR product is suspected.
- Use disposable bench paper to cover work areas.
- All equipment found in the Amplification Area is dedicated to that area and will never be removed unless proper decontamination procedures have been employed.
- Store amplified DNA in the designated amplified DNA freezer. Under no circumstances should the amplified DNA be stored within the DNA Extraction Area.

### 3.3.7 DNA INTERPRETATION

- DNA interpretations must be documented in the analytical record.
- CODIS sheets for evidence samples must be part of the analytical record.

## 3.4 DNA SAMPLE STORAGE

### 3.4.1 EXTRACTED DNA

Extracted DNA will be stored on a short-term basis in a dedicated freezer in the DNA Extraction Area. DNA extracts should be stored in closed containers or sealed bags.

Extracted DNA will be stored long-term frozen in the Property Room. A new barcode number must be created for extracted DNA samples. All new items in File-on-Q require an incident number to be assigned. Any new incident number assigned to extracted DNA samples will follow the format of: "ExtDNA<sup>a</sup>analystthreeletterinitialsMMDDYY".

### 3.4.2 AMPLIFIED DNA

Amplified DNA samples will be stored in a dedicated freezer in the Amplification Area. Under no circumstances will amplified DNA be stored outside the Amplification Area.

Amplified DNA samples will be maintained for a period of six months from the date of amplification. After this time, the amplified DNA will be discarded. Care must be taken to avoid contaminating other areas or evidence with amplified product.

# Unit Policy Manual

## Forensic Biology

### 3.5 POLICIES REGARDING FREEZER PACKETS

Freezer packets are created to store derivative evidence separated or prepared from other evidence that is customarily stored unfrozen.

Anytime a freezer packet is made for derivative evidence the following information (at minimum) should be clearly marked:

- Incident number or Case number (if available)
- Barcode number(s) of the evidence contained within

Any derivative evidence (stains, cuttings, or swabs) that will be impounded into the Property Room as additional evidence will require the generation of a new item in the barcode system under the appropriate incident number. Documentation of the newly generated items should be included in the case notes.

When items that already have a barcode number (or a sub-designation from an existing barcode number) are packaged together, and the packaging is given a new barcode number for the purposes of storage, the barcode of the packaging is for temporary storage purposes and does not become the designator for the items inside.

### 3.6 UNIQUE IDENTIFIERS

General:

Items stored in the Property Room or Laboratory Vault should have a unique identifier. The barcode number uniquely identifies each item stored in the Property Room or Laboratory Vault under the File-on-Q system. Items stored under the old system will be uniquely identified by the case number (or lab sequence number), property tag number, and item number. Multiple items contained within a single package (e.g., the contents of a SART kit) should all be given a sub-item designation, which should be noted in the barcode system for that item.

Any stains, cuttings, or swabs taken of items that will be impounded into the Property Room as additional evidence will require the generation of a new item in the barcode system under the appropriate incident number.

All items included on a laboratory request to be examined by the laboratory (excluding internally produced requests) are considered evidence. All cell pellets, extracted DNA, and amplified DNA will be considered the work product of the DNA laboratory.

During the screening and DNA analysis of evidence it is common to have samples contained in microcentrifuge tubes. These include question stains, reference samples, cell pellets being preserved for possible DNA analysis,

# Unit Policy Manual

## Forensic Biology

and extracted DNA. Each sample analyzed within a batch will be labeled with an identifier that will uniquely identify it during its analysis. Additional descriptors may be employed to identify particular samples such as “NS” to identify non-sperm fractions of extracts, or (Y) to denote a Y-screen extract.

### 3.7 DISCONTINUING ANALYSIS OF SAMPLES

Variables, such as the existence of other probative samples, or the case scenario may affect the decision to discontinue the analysis of a sample. Quantification information may provide additional insight into the likelihood of obtaining interpretable DNA results, and the decision to discontinue analysis may best be made after quantification of the sample(s).

In general, analysts may discontinue analysis of samples under the following circumstances:

If a sample contains insufficient DNA to reasonably obtain an interpretable DNA profile, it may be discontinued. An analyst may be reasonably sure that the sample has insufficient DNA to produce a full DNA profile if a large enough proportion of the sample has been quantified, such that, based on the estimated amount of DNA in the rest of the sample, amplification of the entire sample is unlikely to yield an interpretable DNA profile. Discontinuation of samples is dependent on circumstances of the case, as sometimes even limited information may be beneficial to the investigator(s).

The intent of this policy is to be able to discontinue analysis on single samples from items/cases that do not have enough DNA to obtain a DNA profile. In cases where multiple samples from the same item are collected for the same purpose (e.g. wearer DNA), if one sample has sufficient DNA additional samples collected for the same purpose may be discontinued.

#### Child Abuse Cases

Prior to discontinuing analysis on Child Abuse cases, the most potentially probative evidence item(s) negative for male DNA require microscopic examination to confirm no semen is present.

#### Sexual Assault Cases

If microscopic examination of a sample leads to an estimate that less than 100 total sperm cells are present, the sperm fraction likely does not possess enough DNA to produce a complete autosomal DNA profile and may be discontinued. Given the correlation between the amount of DNA in a sample and the likelihood of obtaining interpretable DNA, the decision to discontinue analysis may best be made after quantification of the samples.

If the sperm fraction of the sample is probative, both the sperm and non-sperm fractions should be continued through DNA analysis.



# Unit Policy Manual

## Forensic Biology

If the non-sperm fraction is probative and sufficient sperm cells are present, both the sperm and non-sperm fractions must be DNA typed.

If the non-sperm fraction is probative and insufficient or no sperm cells are found in the sperm fraction, only the non-sperm fraction needs to be typed.

If the non-sperm fraction is not probative, and its sperm fraction contains insufficient or no sperm cells, the analysis of both fraction can be discontinued.

In sexual assault cases the male DNA is often the probative portion of the sample and the female DNA has little probative value. In such samples, if the estimated amount of male DNA is less than 30pg, the samples are unlikely to yield interpretable DNA results and the sample may be discontinued.

### Firearms Evidence

Swabs from firearms generally have multiple swabs for handler DNA including both “before” and “after” processing swabs. If multiple swabs yield sufficient DNA for DNA typing, then all with sufficient DNA will be DNA typed. If all swabs have insufficient levels of DNA to reasonably obtain an interpretable DNA profile, the swabs will be combined for DNA analysis. If at least one of the swabs has sufficient DNA and the others have an insufficient amount of DNA, the one(s) with insufficient DNA may be discontinued.

### 3.8 INSTRUCTIONS FOR PROCESSING FIREARMS FOR DNA

#### OPTION A: SWABBING FOR DNA FIRST

The Forensic Biology Unit may process guns for DNA prior to latent print processing. In this instance the textured areas of the firearms such as grips, slide, magazine release, safety, trigger, hammer, cylinder release, and slide lock(s) will be swabbed. Smooth areas will be preserved for fingerprint processing.

A sticker will be placed on the packaging indicating that swabbing for potential DNA evidence has been done.

#### OPTION B: FINGERPRINT PROCESSING FIRST

In instances where the majority of the firearm has smooth surfaces, the Crime Scene Unit will process the firearm prior to swabbing for potential DNA evidence.

# Unit Policy Manual

## Forensic Biology

### 4.0 CODIS

The Combined DNA Index System (CODIS) is a nationwide forensic DNA database managed by the FBI. It consists of DNA databases at the local (LDIS), state (SDIS), and national (NDIS) levels. The purpose of CODIS is to develop investigative leads by matching DNA profiles of two or more samples from these various categories. The database primarily consists of casework evidence profiles and convicted offender profiles. Sample categories include Forensic Unknowns (single source profiles), Forensic Partials (single source, but partial profiles), Forensic Mixtures, Convicted Offenders, Arrestees, Suspects, Unidentified Human Remains, Missing Persons, and Relatives of Missing Persons.

The CODIS software searches for genotypes that are represented by the list of alleles uploaded at each locus. Not all genotypes represented by the listed alleles may be accepted based on the interpretation of the evidence. For example, if four alleles at a locus are submitted to CODIS from a 2-person mixture, the search will return hits against any homozygote genotype represented by the listed alleles. Based on the interpretation of a 2-person mixture, any homozygote genotype would be excluded as a possible contributor, even though CODIS would return a hit to it. The case analysts are responsible for determining what is being searched in CODIS in their assigned cases and what genotypes would be acceptable at each locus.

With complex mixtures consultation with the CODIS Administrator is encouraged. If the CODIS Administrator is consulted, documentation of the consultation will be made on the CODIS sheet; however, the technical reviewer is still mandated to assess the types and the sample itself as being acceptable for CODIS. Technical reviewers will document this review by initialing and dating the CODIS sheet. Any material changes made to the CODIS sheet as a result of the technical review must be documented. Any material changes made by the CODIS Administrator after technical review is complete must be discussed with case analyst and documented within the analytical record.

#### 4.1 ENTRY OF DNA PROFILES INTO CODIS

The SDPD Forensic Biology Unit's procedures for the entry of DNA profiles into CODIS are based upon the guidelines outlined in the NDIS Procedures and the California Penal Code.

Many different situations may be encountered in forensic casework that will influence which DNA profiles, or what portion of a DNA profile, are acceptable for entry into CODIS. The following are meant as guidelines and do not cover every possible scenario.

- DNA profiles selected for entry into CODIS are submitted on forms referred to as CODIS sheets. These must be technically reviewed to ensure accuracy of the DNA types and specimen category, and acceptability for CODIS entry. If multiple items from the same case have the same DNA profile, only one CODIS sheet should be used.
- Forensic mixture and forensic partial DNA records submitted to NDIS shall be reviewed to ensure the DNA records satisfy a statistical threshold for match rarity of approximately one in the size of the NDIS database.

# Unit Policy Manual

## Forensic Biology

- DNA profiles (single source or mixtures) from possible perpetrators derived from evidence should be entered into CODIS, unless that evidence was collected from a suspect. In addition to the general eligibility requirements for DNA profiles outlined by NDIS guidelines, the suitability of any given DNA profile for submission to the various levels of CODIS is generally dependent on the discrimination power of the DNA profile as compared to the number of DNA profiles contained within the given database.
- DNA profiles or portions of DNA mixtures attributable to victims or elimination reference samples (boyfriend, husband, etc.) should not be entered into CODIS. Forensic profiles that are ultimately linked to consensual partners will be removed from the database.
- CODIS sheets should be filled out for suspects that were not matched to an evidence sample or that were included as possible contributors to a DNA mixture. These profiles are considered “Suspect Known” DNA profiles. DNA profile information will be entered into the local CODIS database (LDIS) for any suspect (or possible suspect) sample submitted for DNA analysis. These DNA profiles will be maintained indefinitely within LDIS.
- Upon confirmation of their status as a suspect by the submitting detective, “Suspect Known” DNA profiles will be uploaded to the State CODIS Database (SDIS). Suspect Known DNA profiles will remain at SDIS for as long as they are permitted under SDIS regulations.
- In general, CODIS sheets will be removed from the case file during the administrative review and given to the CODIS Administrator for entry. Copies of CODIS sheets must be retained within the analytical record as numbered pages.
- CODIS profiles will be reviewed for eligibility, correct DNA types, and appropriate specimen category prior to upload or search of SDIS.
- Searches of profiles in the local DNA database may be done prior to technical review of the case file.

### 4.2 DATABASE SEARCHES AND MATCHES

Searches of the local DNA database can occur prior to technical review and are also performed at the time of the entry of the profile into CODIS. Searches of the state database occur once per week and are performed by the state (California Department of Justice). Searches of the national database occurs twice a week and are performed by the (FBI) laboratories. DNA profiles are uploaded to the state database at least once a week to coincide with database searches and with the uploading of DNA profiles from state to national databases.

Periodically, information contained within the San Diego Police Department’s (SDPD’s) LDIS may be shared with accredited forensic DNA laboratories in other jurisdictions. Similarly, information from the LDIS of accredited laboratories from other jurisdictions may periodically be shared with our laboratory. In these instances, a “batch file” containing the information within LDIS will be provided for the purpose of comparing DNA profile information between jurisdictions. When the SDPD Crime Laboratory receives a batch file from another agency, the batch file is used for a search against LDIS. The data from the external agency is not stored with the SDPD’s LDIS and ownership of the data is retained by the agency from which it originated. It is the understanding of our laboratory that outside agencies will take the same approach with our LDIS information.

# Unit Policy Manual

## Forensic Biology

There are two basic types of database matches that could provide an investigative lead: forensic hits and offender hits. Forensic hits involve the matching of Forensic Unknowns from two cases. Offender hits involve the matching of a Forensic Unknown to a Convicted Offender.

Any potential database matches will be evaluated by the CODIS Administrator (or his designee). In general, if the SDPD sample matching in CODIS is from an unknown source, the match will be evaluated for “goodness of fit”. If the potential match is deemed to be good, at that time the notification process will be allowed to proceed.

The matching of two evidence samples known or suspected to be from the same person is referred to as a benchwork match. When an evidence sample has already been linked to a suspect and then matches the same person who is in the database as a Convicted Offender sample, this is referred to as a conviction match.

Information regarding forensic and offender hits must be included in at least one report. Prior to a match report being written, investigators may be contacted regarding DNA matches. However, care must be taken to communicate the information accurately, and any significant communication should be documented on a communication log.

### **4.3 MAINTENANCE OF A STAFF INDEX IN CODIS: SAMPLES FROM LABORATORY EMPLOYEES AND INTERNS**

Section 2.9 in the Quality Assurance Manual addresses this database. The following are the rules for the collection and analysis of these samples:

1. A SDPD Reference Mouth Swab Kit will be used to collect two swabs from the individual. The Quality Assurance Manager will coordinate sample collection and coding of the sample.
2. SDPD Reference Mouth Swab Kits from Staff and Interns for which permission to retain has been obtained will be archived with the Quality Assurance Manager.
3. The samples to be analyzed by a qualified DNA analyst following the protocol used to analyze reference mouth swabs in casework.
4. The DNA Technical Manager or CODIS Manager will review the analysis and enter the DNA profiles in the Staff Index of the local SDPD CODIS database.
5. The electropherograms and CODIS printout will be maintained for each sample.

### **4.4 REPORTING DNA RESULTS MATCHING LABORATORY STAFF.**

When an evidence DNA profile is found to match a staff member of the laboratory the following will be done to address the situation:

# Unit Policy Manual

## Forensic Biology

The CODIS match detail report indicating the match to in the coded sample from the staff database will be included in the analytical record.

If the DNA profile in the case was found to match (or the mixture includes) DNA from any staff member in the database, the report will state DNA from staff was found to match/be included to the DNA profile in the case. The report will also address whether any interpretations were affected by the presence of this DNA.

A Staff Match Summary Sheet will be completed detailing the match and will include the coded sample number from the staff database that matched to the evidence.

The Staff Match Summary Sheet will be submitted to the DNA Technical Manager and will be routed through the Quality Assurance Manager. The Quality Assurance Manager will conduct further inquiry into the root cause of the contamination.

### 4.5 OUTSOURCING

In order for the SDPD Crime Laboratory to accept outsourced data for the purposes of searching the CODIS databases the following criteria must be met prior to the initiation of analysis by the vendor laboratory:

- The vendor lab selected for the analytical testing must be accredited and the documentation of that accreditation, including the vendor laboratory's latest external audit report including responses and follow-up must be provided to the SDPD Crime Laboratory.
- Prior approval by the DNA Technical Manager of the technical specifications of any DNA analysis to be performed by the vendor laboratory is required.
- For any new outsourcing agreement an on-site visit to the vendor laboratory by the DNA Technical Manager, or another qualified analyst, is required.

#### 4.5.1 ON-SITE VISITS TO VENDOR LABORATORIES

For any new outsourcing agreement, the DNA Technical Manager, or another qualified (or previously qualified) analyst designated by the DNA Technical Manager or Supervising Criminalist, will perform an on-site visit of a vendor lab prior to the initiation of analysis on the outsourced samples.

An on-site visit to a vendor laboratory shall include at a minimum:

- An evaluation of the laboratory facilities to ensure the integrity of the evidence will be maintained while in the custody of the vendor lab.
- An evaluation of the procedures as they relate to ensuring minimal loss or contamination of the evidence while in the custody of the vendor lab.
- An evaluation of any Corrective Actions taken by the laboratory in the previous six months prior to the on-site visit.

# Unit Policy Manual

## Forensic Biology

- An interview with the vendor laboratory's DNA Technical Manager or laboratory analyst.

The on-site visit will be documented in a report that will be maintained by the DNA Technical Manager.

If an outsourcing agreement with a vendor lab is continued from one year to the next, then an on-site visit to the vendor laboratory by another NDIS participating laboratory using the same technology, platform, and typing test kit may be used. Documentation of the on-site visit by a second NDIS participating laboratory must be obtained by the SDPD Crime Laboratory before any new analysis is initiated in the new year of the agreement.

### 4.5.2 REVIEW OF OUTSOURCED DATA

Prior to uploading outsourced data into the databases of the Combined DNA Index System (CODIS) a technical review of the data will be performed. The review of the data will be performed by a qualified (or previously qualified) DNA analyst and will be documented and include:

- A review of the analytical record to include all controls (ladders, positive and negative amplification controls, and blanks).
- A review of the vendor laboratory's final report to ensure that the results/conclusions are supported by the data, and that each tested item (or its probative fraction) is addressed.
- A review of the data for all submitted samples to the vendor laboratory.
- A verification of the DNA types, eligibility, and correct specimen category of any sample to be uploaded to the CODIS databases.

# Unit Policy Manual

## Forensic Biology

### 5.0 LABORATORY MAINTENANCE

#### 5.1 GENERAL LABORATORY MAINTENANCE

All members of the Forensic Biology Unit are responsible for the general upkeep of the laboratory including stocking supplies, washing dishes, and cleaning common areas. Protective gloves, safety goggles, and a laboratory coat should be worn when handling chemicals and cleaning lab instruments. The following tasks are completed each week:

- Monitor the operating temperatures of all freezers, refrigerators, and incubators. Log temperatures on the log sheets. When a sheet has been completed, it should be scanned into the FB network and moved to the appropriate folder within the QA-QC folder. The scanned sheet should be saved using a file name that clearly indicates what the log is for.
- Wipe general use counters with 10-20% bleach solution.
- Clean common use centrifuges with 10-20% bleach solution followed by wiping with distilled water.
- Wipe hoods with 10-20% bleach solution (do not wipe plastic doors).
- Clean interior and exterior of drying boxes with 10-20% bleach solution.
- Wash glassware and dishes not belonging to an analyst (analysts are responsible for their own dishes). Return washed dishes to their appropriate storage location.
- Fill distilled water carboys, reagent alcohol bottles, 10-20% bleach solution bottles, and Liquinox detergent bottles.

The above tasks apply to all rooms of the Forensic Biology Unit. Every two weeks, the Amplification Room floor will be mopped with 10-20% bleach solution. Record the completion of maintenance tasks in the Main Forensic Biology Unit Maintenance Log, and Amplification Room Maintenance Log.

##### 5.1.1 Glassware Washing

It is the responsibility of each analyst to wash their own dishes. Protective gloves, safety glasses, and a lab coat or apron should be worn.

1. Wash glassware in warm water with Liquinox detergent.
2. Rinse detergent from glassware by rinsing in tap water.
3. Rinse each piece of glassware in distilled water.

# Unit Policy Manual

## Forensic Biology

4. Set glassware to dry.
5. Return glassware to appropriate storage location when dry.

Note: The most critical concern about glassware in the DNA Laboratory is that glassware in the Amplification room and glassware from the DNA Extraction-PCR Setup Area be kept separate. The use of glassware dedicated to the Amplification area in the DNA Extraction-PCR Setup area is far more serious than the reverse. The only circumstance in which glassware may be transferred from the Amplification Room to the DNA Extraction-PCR Setup Area is if the glassware has been treated with bleach for 30 minutes followed by autoclaving.

### 5.2 CLEANING LOGS

The following cleaning logs will be maintained by the Forensic Biology Unit. They are intended to ensure common use areas are kept in a clean state. Any cleaning should be entered electronically into the FB cleaning log excel workbook.

Cleaning Log	Location	Updated
Forensic Biology Main Lab	Electronic	Weekly
Amplification Room	Electronic	Weekly/ Biweekly (floors)
Qiagen BioRobot® EZ1	Electronic	Monthly

### 5.3 CLEANING AND DECONTAMINATION PROCEDURES

Squirt bottles containing a dilute bleach solution (10-20%) are present in the different work areas in the DNA Laboratory. Following DNA extraction, PCR setup, or preparation of amplified DNA samples the work areas that were utilized will be thoroughly washed with the bleach solution making sure the entire work areas are treated.

If lab bench paper has been laid down and is thought to be contaminated with DNA, the lab bench paper should be disposed of.

Racks or glassware thought to be contaminated with amplified DNA will be treated in the manner described below.

For items that cannot be autoclaved, they will be soaked in 20% bleach for 30 minutes, rinsed thoroughly with tap water, rinsed in distilled water, and allowed to air dry.



# Unit Policy Manual

## Forensic Biology

For items that can be autoclaved, they will be treated first with bleach as previously described and then autoclaved for thirty minutes.

### 5.3.1 DECONTAMINATION OF CENTRIFUGES

In general, any removable part of a centrifuge can be cleaned with detergent and water, rinsed with distilled water, and allowed to dry. If a serious contamination problem occurs (i.e. spilled extracted or purified DNA), the rotor should be rinsed in a 10-20% bleach solution, rinsed with distilled water, and allowed to dry.

The metal tubes used with the Sorvall centrifuge should be cleaned with detergent and water after each use. The metal rotors and tubes the Sorvall centrifuge can also be autoclaved.

### 5.4 DISPOSAL OF HAZARDOUS WASTE

There are six waste streams within the Forensic Biology Unit that should be handled as hazardous waste. These are as follows:

#### 5.4.1 PHENOLPHTHALIN CONTAMINATED SWABS

Phenolphthalin contains ethanol and swabs contaminated with it will go into appropriately labeled waste buckets. After the swabs have dried the swabs can be disposed of in the regular trash.

#### 5.4.2 PHENOLPHTHALIN AND LMG LIQUID WASTE

Phenolphthalin and LMG liquid waste contains zinc; a heavy metal. The unusable liquid waste will be stored in the Chemical Storeroom for disposal. The bottles should be appropriately labeled with the hazard (flammable for pheno and corrosive for LMG). The zinc contained in both reagents should also be noted on the log.

#### 5.4.3 QIAGEN REAGENT WASTE (EZ1 or QiaSymphony)

EZ1 strips are flammable because of the ethanol they contain and should be placed in the designated waste containers by the instruments. All tips, tip holders, and tubes can be disposed of as regular trash. When the EZ1 strip waste containers are full the waste can be transferred to the Chemical Storeroom for disposal. Liquid QiaSymphony waste can also be transferred to the Chemical Storeroom.

#### 5.4.4 PHENOL-CHLOROFORM-ISOAMYL ALCOHOL WASTE

Phenol-chloroform-isoamyl alcohol is an organic solvent and the liquid waste goes in the red waste containers in the hoods. Tips and tubes contaminated with phenol-chloroform-isoamyl alcohol should be allowed to evaporate in the hood. After evaporation the plastic ware can be disposed of in the regular trash.

# Unit Policy Manual

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## Forensic Biology

### 5.4.5 FORMAMIDE WASTE

Formamide is a known teratogen and the formamide waste will be disposed of in the waste bucket in the amplification room. When full the waste can be transferred to the Chemical Storeroom for disposal.

### 5.4.6 BIOHAZARDOUS WASTE

Large volumes of liquid blood are biohazardous. Any large volumes of blood and the materials used to clean them up should be disposed of in red biohazard containers.

### 5.4.7 NON-HAZARDOUS WASTE

Small volumes of blood that will dry within a reasonable amount of time can be disposed of in the regular trash. LMG test swabs can be disposed of in the regular trash. All plasticware [tips, tubes (including microcon/centricon tubes), transfer pipets, etc] and gloves can be disposed of in the regular trash. Acid phosphatase test swabs can be disposed of in the regular trash and any waste liquid down the drain.

Archived Version

# Unit Policy Manual

## Forensic Biology

### 6.0 EQUIPMENT MAINTENANCE PROTOCOLS

#### 6.1 USE AND MAINTENANCE OF THE WATER SYSTEM

The ultra/nano pure system uses the distilled water reservoir as its water source.

##### **Ultra pure water**

The Barnstead NANOpure ultra pure water system removes ions from water. It is meant to be used with pretreated water. Hence, the water used is first distilled. The system can be set to three different modes: off, standby, and on. The system is left on standby when not actively in use to allow water to be circulated through the cartridges for five minutes out of every hour. This extends the life of the cartridges. When ultra pure water is needed, set the system to the on mode by pressing the 'ON/STANDBY/OFF' button until a numerical value appears. This number represents a resistivity measurement of the water, which indicates its purity. Wait for the number to increase to at least 18.0 before dispensing. If this number remains below 18.0, then the cartridge is no longer efficiently deionizing the water. A carboy is used to collect and store ultra pure water, as the system produces purified water rather slowly. The ultra pure water system should never be used when the distilled water reservoir is empty. A low water protection device has been built into the distilled water reservoir to prevent the ultra pure water system from operating when the still is low.

#### 6.2 OPERATION OF THE pH METER

Thermo Scientific Orion Star A111 pH Meter Operation

##### Electrode Preparation

1. Disconnect electrode from meter.
2. Uncover the filling hole and add electrode filling Ag/AgCl solution.
3. Solution must be above reference junction and at least one inch above sample level.
4. **The filling hole must be open when electrode is in use and closed when in storage.**
5. Place the electrode in the electrode holder and suspend it in air for 5 minutes so that the solution in the electrode can thoroughly wet the reference junction.
6. Shake electrode downward (similar to a thermometer) to remove air bubbles.
7. Soak electrode in pH electrode storage solution for 30 to 60 minutes.
8. Connect electrode to meter.

##### Electrode Storage

1. Short-term Storage (up to one week)
  - a. Soak the electrode in pH electrode storage solution.
  - b. To prevent crystallization of the fill solution, cover the fill hole whenever the electrode is being stored and open the fill hole when calibrating and measuring.
2. Long-term Storage (more than one week)
  - a. Fill the reference chamber and securely cover the filling hole.

# Unit Policy Manual

## Forensic Biology

- b. Cover the sensing element and reference junction with the protective cap/sleeve containing a few drops of storage solution.
- c. Before returning the electrode to use, prepare it as a new electrode.

### Electrode Maintenance

1. Inspect the electrode for scratches, cracks, salt crystals.
2. Rinse off salt crystals with DI water.
3. Drain the reference chamber, flush it with fresh filling solution and refill the chamber with fresh filling solution.

### Electrode Cleaning

1. Soak electrode in 0.1 M HCL or HNO<sub>3</sub> for 30 min.
  - a. Can also be soaked in a 1:10 dilution of household bleach mixed with hot water (stir at fast rate).
2. Drain the reference chamber and refill it with fresh filling solution.
3. Soak the electrode in pH electrode storage solution for at least 1 hour.

### Calibration

1. Prepare the electrode according to the electrode preparation instructions.
2. Connect the probe to the meter and turn on the meter.
3. Press **mode** to display pH for the pH measurement mode.
4. Select 2 pH buffers that bracket the pH you are targeting.
5. Press **Cal**
6. Rinse the electrode in DI water and blot with a kim wipe.
7. Place electrode into first buffer.
8. Wait for **“Ready”** then record value in log book.
9. Rinse the electrode in DI water and blot with a kim wipe.
10. Place electrode into second buffer.
11. Wait for **“Ready”** then record value in log book.
12. Rinse the electrode in DI water and blot with a kim wipe.

### Measurement

1. Perform Calibration steps first.
2. Make sure it is in pH measurement mode.
3. Press **measure**: the meter will immediately start taking readings.
4. When **“Ready”** is displayed the meter stops measuring the pH, so you may need to press **measure** multiple times when pH-ing the solution.
5. When you reach the target pH rinse the electrode in DI water and blot with a kim wipe.
6. Turn pH meter off.

Store electrode according to the Electrode Storage directions provided.

## 6.3 OPERATION OF THE AUTOCLAVE

The autoclave is used to sterilize critical reagents and equipment via steam pressurization. There are two exhaust selector settings on the autoclave: FAST (Instruments) and SLOW (Liquids). Use the slow setting when only liquids or liquids and equipment are being sterilized. If sterilizing equipment use the fast setting.

# Unit Policy Manual

## Forensic Biology

1. Close the drain valve.
2. Fill with autoclave with approximately 5.5 liters distilled water and 0.5 liters tap water.
3. Load autoclave with items to be sterilized. Place autoclave tape on lids, if necessary, and be sure lids are on loosely.
4. Close autoclave door. Be sure the door closes tightly. If not, adjust the screw below the door handle latch.
5. Set the exhaust selector to "Fast or Slow", depending on the items being sterilized.
6. Set the time for 30 minutes.
7. When the sterilization is complete, carefully open the door and drain valve. Do not open the autoclave immediately after sterilization is complete due to the high heat and pressure; allow to cool and depressurize.

### 6.4 BALANCES

Balances and microscopes in the Forensic Biology Unit will be maintained and the calibration checked according to policies outlined in the laboratory Quality Assurance Manual. Laboratory balances are calibrated by an outside company once a year.

The calibration is checked every three months by the Laboratory Technician. Balance calibration is checked by weighing various NSB certified weights, given in the tables below. The acceptable range of variation is 1% from the true value for weights less than one gram, and 5% from true value for weights greater than one gram.

If a balance is found to be out of calibration, it has to be sent to an outside company to be recalibrated. Once returned, the calibration of the balance will be checked by the Laboratory Technician before use in the laboratory.

Analytical Balance	
Weight (g)	Acceptable Range (g)
0.01	0.0099 - 0.0101
0.05	0.0495-0.0505
0.10	0.0990-.0.1010
0.50	0.4950-0.5050
1.00	0.9500-1.0500
5.00	4.7500-5.2500
10.00	9.5000-1.0500
50.00	47.5000-52.5000
100.00	95.0000-105.0000

Pan Balance	
Weight (g)	Acceptable Range (g)
1.00	0.95-1.05
5.00	4.75-5.25
10.00	9.50-10.50
50.00	47.50-52.50
100.00	95.00-105.00
200.00	190.00-210.00

# Unit Policy Manual

## Forensic Biology

### 6.5 MAINTENANCE OF THE QIAGEN EZ1s

Each month the piercing units are cleaned and the O-rings are greased on the Qiagen EZ1 robots. Maintenance actions are recorded on the Qiagen EZ1 Maintenance Worksheet in the Qiagen EZ1 Maintenance Log. A volume test is conducted every six months to monitor the pipetting accuracy of the robots. Results of the volume test are recorded on the BioRobot EZ1 Volume Test Worksheet in the Qiagen EZ1 Maintenance Log.

#### 6.5.1 CLEANING THE PIERCING UNIT:

The piercing unit cleaning protocol can be accessed using either the DNA Investigator Card or the Volume Test Card. If only a cleaning is required, use the DNA Investigator Card; if a volume test is also due, then use the Volume Test card. This will limit the number of times the machine is turned on and off and cards are switched. **Never remove or insert a card when the machine is on.** The DNA Investigator Card should always be in the machine so that it ready for use.

1. With the robot off, insert the Volume Test Card or ensure that the DNA Investigator Card is in the card slot.
2. With the door closed, turn the machine on.
3. Press **1. Protocols**.
4. Press **3. Clean piercing unit**. The piercing unit will move forward and downward.
5. Open the door by lifting up, and clean the piercing unit with reagent alcohol.
6. Close the door and press **Esc** when finished.

#### 6.5.2 GREASING THE O-RINGS:

Apply a small amount of silicon grease to the top of a filter tip and use it to apply the grease to the tip adapters. Wipe off any excess grease with a tissue.

#### 6.5.3 VOLUME TEST:

The volume test requires the use of the Volume Test Card.

1. With the machine turned off, insert the Volume Test Card.
2. For each robot to be tested, individually number and weigh six empty elution tubes with caps. Record the weight of the empty elution tubes on the BioRobot EZ1 Volume Test Worksheet in the Qiagen EZ1 Maintenance Log.

# Unit Policy Manual

## Forensic Biology

3. Place the six empty elution tubes, without their caps, in row 1.
4. Place six sample tubes containing 1mL of distilled water in row 4.
5. Place six tips in tip holders in row 2.
6. With the door closed, turn the machine on.
7. Press the **Start** button. The volume test will begin.
8. When the test is complete, press **Esc** to exit the protocol and turn the machine off.
9. Remove the Volume Test Card and insert the DNA Investigator Card.
10. Replace the caps on the elution tubes and remove them from the machine.
11. Weigh each tube, now filled with approximately 500  $\mu$ L of distilled water.

Record the weights of the water on the Volume Test Worksheet and calculate the variance. Allowable variance is within 5% of the volume pipetted. For the 500 $\mu$ L pipetted in the volume test, the allowable range is 474 to 525  $\mu$ L. Recall that 500 $\mu$ L is equal to 500mg.

### 6.6 HAMILTON NIMBUS 4 MAINTENANCE

The Nimbus requires routine maintenance to be performed daily, weekly, and semi-annually in order to ensure optimal performance. Any maintenance failures should be reported to Hamilton Technical Support (1-800-648-5950) for troubleshooting assistance.

#### Daily Maintenance:

The daily maintenance should be performed prior to the first run of the day. It is not necessary to perform the daily maintenance prior to subsequent runs if it has already been performed on a given day. It is also not necessary to perform the daily maintenance on days the Nimbus is not used.

1. Power on the Nimbus instrument and attached computer.
2. Open the Maintenance software (on the desktop).
3. Select the “**Daily**” maintenance type and click “**Run Maintenance**”.
4. Inspect deck and carriers for cleanliness.

# Unit Policy Manual

## Forensic Biology

- a. Clean the deck with deionized water and/or ethanol.
  - b. Wipe the o-rings on the channels with deionized water.
  - c. Check the **“Inspect deck and carriers for cleanliness”** box.
5. Empty Tip Waste/Liquid Waste.
- a. Remove the tip waste receptacle and empty tips into trash.
  - b. Replace plastic trash bag in tip waste receptacle if needed.
  - c. There is no liquid waste to empty.
  - d. Check the **“Empty Tip Waste/Liquid Waste”** box.
6. Select the **“Check tightness of Pipetting Channels”** box.
- a. Read the message that appears, remove the tip eject plate, close the door, and click **“Continue”**.
  - b. The system will automatically test the o-ring seals of the channels.
7. Select the **“Check LLD”** box.
- a. Read the message that appears, ensure the tip eject plate is still removed and the door is still closed, and click **“Continue”**.
  - b. The system will automatically test liquid level sensing.
8. Click **“OK”** to acknowledge that the maintenance has completed successfully.
9. Replace the tip eject plate.
10. Click **“Close”**.
11. Exit the Maintenance software.

### Weekly Maintenance:

The weekly maintenance should be performed once a week regardless of whether or not the Nimbus is to be used that week.

1. Power on the Nimbus instrument and attached computer.
2. Open the Maintenance software (on the desktop).
3. Select the **“Weekly”** maintenance type and click **“Run Maintenance”**.
4. Clean deck and carriers.
  - a. Clean the deck with deionized water and/or ethanol.
  - b. Check the **“Clean deck and carriers”** box.
5. Check condition of carriers and other devices.
  - a. Visually inspect the instrument for any obvious signs of damage.



# Unit Policy Manual

## Forensic Biology

- b. Clean the pedestals and racks with water and/or ethanol.
  - c. Check the **“Check condition of carriers and other devices”** box.
6. Empty and clean Tip Waste/Liquid Waste.
    - a. Remove the tip waste receptacle and empty tips into trash.
    - b. Replace plastic trash bag in tip waste receptacle if needed.
    - c. There is no liquid waste to empty.
    - d. Clean the tip eject plate with deionized water and/or ethanol.
    - e. Check the **“Empty and clean Tip Waste/Liquid Waste”** box.
  7. Clean each channel: stop disk, o-ring, and tip eject sleeve.
    - a. Wipe the pipettor channels with a kimwipe dampened with deionized water.
    - b. Check the **“Clean each channel: stop disk, o-ring, and tip eject sleeve”** box.
  8. Clean the side covers
    - a. Clean the Nimbus enclosure with deionized water and/or ethanol.
    - b. Check the **“Clean the side covers”** box.
  9. Select the **“Check tightness of Pipette Channels”** box.
    - a. Read the message that appears, remove the tip eject plate, close the door, and click **“Continue”**.
    - b. The system will automatically test the o-ring seals of the channels.
  10. Select the **“Check cLLD”** box.
    - a. Read the message that appears, ensure the tip eject plate is still removed and the door is still closed, and click **“Continue”**.
    - b. The system will automatically test liquid level sensing.
  11. Click **“OK”** to acknowledge that the maintenance has completed successfully.
  12. Replace the tip eject plate.
  13. Click **“Close”**.
  14. Exit the Maintenance software.

### Semi-Annual Maintenance:

At least once a year a service engineer from Hamilton will perform maintenance and calibration of the Nimbus.

### REFERENCES:

Microlab® Nimbus Independent Channel Operator’s Manual. Hamilton Robotics.  
Microlab® Nimbus Enclosed Version Operator’s Manual. Hamilton Robotics.  
Microlab® Nimbus Independent Channel Programmer’s Manual. Hamilton Robotics.

# Unit Policy Manual

## Forensic Biology

### 6.7 7500 SDS CALIBRATION / VERIFICATION PROCEDURES

The information for the procedures detailed in this section was taken from the Applied Biosystems 7300/7500/7500 fast real-Time PCR System Installation and Maintenance Guide. When the yearly calibration checks are performed, the analyst performing the calibration must check the lamp usage and status. Procedure 6.8.7.1 details checking the lamp usage and status. The halogen lamp located in the 7500 SDS Instrument has a life span of approximately 1,000 hours. If the lamp has exceeded 1,000 hours, or if a message is displayed that recommends changing the lamp, procedure 6.8.7.2 should be followed.

#### 6.7.1 BLOCK CONTAMINATION CHECK

Purpose: A check for contamination allows a user to determine if there are any areas of possible contamination in the wells of the plate holder that would artificially increase the fluorescence in those wells during a run, thereby increasing the quantitation result.

Preparing the Check This check is performed without a plate being positioned in the plate holder; therefore, if there is a plate in the holder, remove it, and then close the carriage door over the sample block and lock it down.

Note: This check is for information only and should not be saved using the 7500 System software during or after the check. See step 3 below for instructions on documenting the contamination check.

#### Performing the Check

1. Turn on the instrument and open the SDS software. Click **File>New**. In the new document wizard click Finish to accept the default parameters.
2. Using Filter A (FAM filter position), open the ROI Inspector and take an image at 2048ms by selecting **Snapshot**.
3. Observe the background fluorescence in the 96 wells. Note any wells that have significant fluorescence; fluorescence indicates contamination. For documentation, perform a Screen Shot (**Alt + PrintScrn**) and then paste it (**Ctrl-V**) into a Microsoft Word blank document.
4. To determine an acceptable background fluorescence level:
  - a. Push the instrument carriage and allow it to spring back to open it.
  - b. Put a new, clean, empty reaction plate without the cover into the sample block.
  - c. Push the carriage forward to close it.
  - d. Take another image at 2048 ms. For documentation, perform a Screen Shot (**Alt +PrintScrn**) and then paste it (**Ctrl-V**) into a Microsoft Word blank document.
  - e. Save as Calibration Contamination check (MM/DD/YY) in the appropriate instrument folder.

# Unit Policy Manual

## Forensic Biology

5. If some of the wells still appear to have significant fluorescence, clean them per the instructions in “Cleaning the Sample Wells”.

Recheck the wells after cleaning, following steps 1 through 3 above.

### 6.7.2 7500 REGIONS OF INTEREST CALIBRATION

#### MATERIALS REQUIRED:

1. Applied Biosystems Real-Time PCR System Spectral Calibration Kit
2. ROI Calibration Plate (Part No. 4349415)
3. Centrifuge and vortex

#### Purpose:

A Regions of Interest (ROI) calibration maps the positions of the wells on the sample block so that the software can associate increases in fluorescence during a run with specific wells of the plate. A calibration image is needed for each well or filter to account for minor differences in the optical path.

#### Preparing the Plate

Retrieve the ROI calibration plate from the spectral calibration kit from the freezer in the amplified DNA room freezer. Allow the plate to thaw. Leave the optical film on the plate. (Do not discard the packaging as the plate can be used up to three times). Vortex and centrifuge the thawed plate to assure that the liquid is in the bottom of the plate wells.

#### Creating a Plate Document for the ROI Calibration

1. Start the instrument and open the SDS software. Load the ROI calibration plate into the plate holder position.
2. Click **File > New**  
In the New Document wizard or dialog box click **Finish** to accept the default parameters. (The SDS software automatically saves the ROI data to a set of calibration files on the computer hard drive). Click OK if any ROI error is displayed.
3. In the SDS software, select **Instrument > Calibrate**
4. In the ROI inspector dialog box that appears, right-click the black area of the window, then select **Display Properties**.
5. In the Image Viewer Control Properties dialog Box:

# Unit Policy Manual

## Forensic Biology

- a. Select **Show Saturation**
  - b. In the Sat Threshold field, enter **4000**.
  - c. Click **OK**
6. In the ROI Inspector dialog box, set the lamp control:
    - a. Click **Block up**
    - b. Select **Idle** from the Lamp Control drop-down list

### Performing the ROI Calibration

1. In the ROI Inspector dialog box:
  - a. In the Exposure Time field, enter **2048** for the 7500 and
  - b. Select **Filter A**.
2. Click **Snapshot** to generate an ROI image.
3. Determine if your ROI image is acceptable. An acceptable image will not have saturated red areas in any wells. (A few red pixels in a well are acceptable).

If the ROI image appears over saturated, continue to reduce the number in the Exposure Time Field (2048) by half until an acceptable image is obtained. Once the acceptable image is produced, print this screen by pressing **Alt + PrintScrn** and then **Ctrl V** into a Microsoft Word blank document.

4. If your image is acceptable, Click **Generate Calibration**.  
The software takes a snapshot, and then displays a message dialog box or an ROI image. A successful calibration will have green circles around all wells.

*If the calibration image is not successful, see pg 45 for of the 7500 Installation and Maintenance Guide for trouble shooting.*

5. Click **Save Calibration** to save the image for filter A. (An OK will appear in the dialog box for Filter A). Remember to **Save** after every filter.
6. Repeats steps 1 through 6 for the remaining filters: Filters B, C, D and E. Reset the Exposure Time to 2048 before performing the calibration for each filter.
7. Click **Done > File > Close** and click **NO** to save the plate document.

### Unloading the Plate

In the ROI Inspector dialog box, click Block Down and remove the plate. Return the plate to the packaging if not performing background and optical calibration.

# Unit Policy Manual

## Forensic Biology

### 6.7.3 Performing the Background Calibration on the 7500 SDS Instrument

#### MATERIALS REQUIRED:

1. Applied Biosystems Real-Time PCR System Spectral Calibration Kit
2. Background Plate (Part No. 4330124)
3. Centrifuge and vortex

**Purpose** A background calibration measures the level of background fluorescence in the instrument. The run will perform continuous reads of a background plate containing PCR buffer for 10 minutes at 60°C and average the spectral results to a calibration file. The software can then use the file to remove the background fluorescence from the run data.

Perform a background calibration after replacing the lamp or monthly, depending on instrument usage.

**Precautions** Since the signal consists of fluorescence from several sources it is important to use gloves when handling the plate, use a clean centrifuge and vortex, and do not place the plates on the lab bench.

**Preparing the Background Calibration Plate** Retrieve the prepared background plate from the spectral calibration kit from the freezer in the amplified DNA room. Allow the plate to thaw (Do not discard the packaging as the plate can be used up to three times). Vortex and centrifuge the thawed plate to ensure that the liquid is in the bottom of the plate wells.

#### Creating a Plate Document for the Background Calibration

1. Place the prepared plate into the plate holder and close the tray/door. Start the instrument and open the SDS software. Click **File > New**.
2. Configure the New Document Dialog box:
  - a. Select **Assay > Background**.
  - b. Select **Container > 96-Well Clear**.
  - c. Select **Template > Blank Document**
  - d. In the Operator field, enter a name.
  - e. In the plate Name field, enter : **Background\_<date MMDDYY>** and click **Finish**.
3. In the SDS software select **File > Save As**. On 7500 #1, navigate to C:\SDS1.2\Documents and save. On 7500 #2, navigate to D\ : > SDS 1.2 > SDS Documents and save. Select **Save as type > SDS Documents (\*.sds)**.

# Unit Policy Manual

## Forensic Biology

Performing the Background Calibration In the SDS software: Select the **Instrument** tab and click **Start**. The instrument begins the calibration run after the heated cover comes to temperature. After the run is complete, click **OK**. The background run may take 10 minutes.

### Analyzing the Background Calibration Data

1. Click the green arrow or select **Analysis > Extract Background**. The software extracts the background signal then displays **Background Extraction Complete**. Click **OK**.

*If the message indicates the image exposure is too low, see pg 56 of the 7500 Installation and Maintenance Guide for trouble shooting.*

2. In the plate document, select the **Results** tab, then **Spectra** tab. Print this page for the Background Calibration records and to show that the instrument is free of significant contaminants.
3. Inspect the raw data for irregular spectral peaks that exceed the following fluorescent standard units (FSU). All wells should be selected.

Filter	FSU
A,B,C,D (7000/7500)	>72,000
E (7500)	>90,000

*If the fluorescence exceeds the FSU indicated, see pg 64 of the 7500 Installation and Maintenance Guide for trouble shooting.*

*If a contaminant is observed (high fluorescent data), see pg 108 of the 7500 Installation and Maintenance Guide for cleaning contaminants.*

4. Inspect the background plate image for bright areas of fluorescence and perform a Screen Shot (**Alt + PrintScrn**) and then paste it (**Ctrl-V**) into a Microsoft Word blank document for documentation.
5. Select **File > Close**

# Unit Policy Manual

## Forensic Biology

### 6.7.4 PERFORMING THE OPTICAL CALIBRATION FOR THE 7500 SDS INSTRUMENT

#### MATERIALS REQUIRED:

1. Applied Biosystems Real-Time PCR System Spectral Calibration Kit
2. ROI Calibration Plate (Part No. 4349415)
3. Centrifuge and vortex

**Purpose:** The optical calibration compensates for the physical effects of the additional filter present in the 7500 instrument and needs to be performed after every 7500 system background calibration.

#### Preparing the Optical Calibration Plate

Retrieve the prepared ROI plate from the spectral calibration kit from the freezer in the amplified DNA room. Allow the plate to thaw (Do not discard the packaging as the plate can be used up to three times). Vortex and centrifuge the thawed plate to ensure that the liquid is in the bottom of the plate wells.

#### Creating a Plate Document for the Optical Calibration

1. Start the instrument and open the SDS software. Load the ROI calibration plate into the plate holder position.
2. Click File>New.
3. Configure the New Document Dialog box:
  - a. Select **Assay >Calibration**.
  - b. Select **Container > 96-Well Clear**.
  - c. Select **Template > Blank Document**
  - d. In the Operator field, enter a name.
  - e. In the plate Name field, enter :  
**Calibration\_<date MMDDYY>** and click **Finish**.
4. In the SDS software select **File > Save As**. On 7500 #1, navigate to C:\SDS1.2\Documents and save. On 7500 #2, navigate to D\ : > SDS 1.2 >SDS Documents and save.

#### Performing the Optical Calibration

In the SDS software: Select the **Instrument** tab and click **Start**. The instrument begins the calibration run (10 min.) after the heated cover comes to temperature. After the run is complete, click **OK**.

#### Analyzing the Optical Calibration Data

Click the green arrow or select **Analysis > Extract**. The software extracts the optical calibration then displays a message indicating the extraction is complete. Click **OK**.

# Unit Policy Manual

## Forensic Biology

Click on the Results and Spectral Tabs. Select all wells and print this screen. Inspect the raw background data for any irregular spectral peaks.

*For any error messages, see pg 64 of the 7500 Installation and Maintenance Guide for trouble shooting*

Click **File>Close**.

### 6.7.5 PERFORMING THE PURE DYE CALIBRATION ON THE 7500 SDS INSTRUMENT

#### MATERIALS REQUIRED:

1. Applied Biosystems 7500 Real-Time PCR Systems Spectral Calibration Kit I (PN 4349180 containing Pure Dye Plates FAM, JOE, NED, ROX, SYBR Green, TAMRA, and VIC).
2. Centrifuge and vortex

Purpose During a pure dye calibration run, the system collects spectral data from a series of pure dye standards and stores the spectral information for the pure dye standards in the pure spectra run file, a calibration file in the SDS directory.

The software then uses the pure spectra data during subsequent runs to characterize pure dyes and distinguish the individual contribution of each dye in the collective fluorescence collected by the instrument during a run.

**Important!** Before performing a pure dye calibration, you must perform an ROI calibration, a background calibration, and an optical calibration.

Preparing the Plate Retrieve the pure dye plates from the spectral calibration kits from the freezer in the amplified DNA room. Allow the plates to thaw (~5 minutes). **NOTE: Do not remove a pure dye plate from its packaging until it is ready to be used. The fluorescent dye in the wells of each pure dye plate is photosensitive.** (Do not discard the packaging as the plates can be used multiple times). Leave the optical film on the plates. Although multiple pure dye plates are in Spectral Calibration Kit I, only the plates that are currently used for the quantitation chemistry being employed need to be run. For Quantifiler and Quantifiler Duo, only FAM, VIC, ROX, and NED need to be run.

#### Creating a Plate Document for the Pure Dye Calibration

1. Start the instrument and open the SDS software. Click **File > New**.
2. In the New Document Wizard dialog box:



# Unit Policy Manual

## Forensic Biology

- a. Select **Assay > Pure Spectra**.
- b. Select **Container > 96-Well Clear**.
- c. Select **Template > Blank Document**.
- d. In the Operator field, enter your initials.
- e. In the Comments field, enter the plate bar code number.
- f. Click **Finish**. Note: It is not necessary to name or save the pure dye plate document. The SDS software automatically saves the pure dye data to a calibration file on the computer hard drive.

### Selecting the Dye

In the Pure Spectra Calibration Manager dialog box:

- a. In the Dye List field, select a pure dye to calibrate.
- b. Click **Calibrate**.
- c. If you are prompted to disconnect the plate document, click **Yes**.
- d. A message prompts you to load the plate. Do not click Yes or No at this point.

### Preparing and Loading a Pure Dye Plate

1. Remove the appropriate Pure Dye plate from its packaging. Do not discard the packaging as the plate can be used multiple times. Leave the optical film on the plate.
2. Vortex and centrifuge the thawed plate to assure that the liquid is in the bottom of the plate wells.
3. Verify that the pure dye plate that you are about to load matches the dye selected in the Pure Spectra Calibration Manager.
4. Load the plate in the instrument with the notch in the upper right corner.

### Performing the Pure Dye Calibration

1. In the dialog box that prompts you to load the plate click **Yes**. The run takes ~5 minutes to complete.
2. When the SDS software completes the run, remove the pure dye plate from the SDS Instrument, place it back into its packaging, and place it back into its respective spectral calibration kit in the freezer.

Calibrating remaining dyes:

1. Click **Next Dye >**.
2. Repeat the procedures in Preparing and Loading a Pure Dye Plate and Performing the Pure Dye Calibration to run the remaining pure dye plates.
3. After you calibrate the instrument with all pure dyes provided in the spectral calibration kits, click **Finish**.

### Analyzing the Pure Dye Calibration Data

# Unit Policy Manual

## Forensic Biology

When each pure dye plate is run, the SDS Software automatically creates and saves a plate document for each dye. After you calibrate all of the pure dye plates, the plate documents remain open behind the plate document displayed by the software. *To complete the calibration, analyze all open pure dye documents:*

1. From the Windows menu, select the plate document to analyze.
2. Select the **Results** tab, then select the **Spectra** tab.
3. Select all wells of the plate document by clicking the upper-left corner of the plate grid.
4. Click the green arrow (or select **Analysis > Extract Pure Spectra**). The SDS Software completes the extraction, then displays a message:

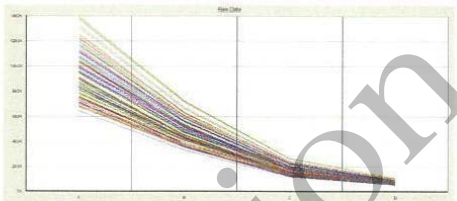
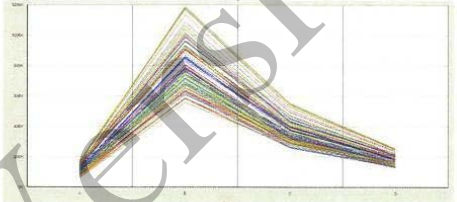
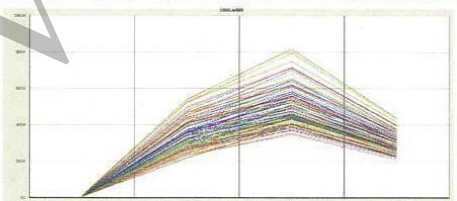
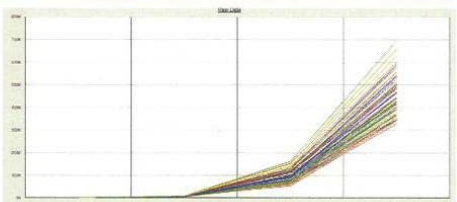
If the software displays:	Do this:
Pure Spectra Extraction Complete – The analysis is successful.	Click <b>OK</b> , then go to step 5. <b>Important! The pure dye calibration is not complete at this point.</b> Before closing the plate document you must inspect the Spectra plot as explained in steps 5 through 8 below.
Repair Message—“The following wells require repair. . .”	Click <b>OK</b> , then go to step 5. (For information on how the software auto-repairs calibration spectra, see “About Pure Dye Spectra” on pg 79 of the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.
Error Message—“Pure Spectra Extraction Detected an Unknown Error. . .”	Click <b>OK</b> , load the plate, then run the pure dye plate again. If the calibration continues to fail, perform the calibration with a new pure dye plate.

5. In the pure dye plate document:
  - a. Select the **Results** tab.
  - b. Select the **Spectra** tab.
6. Select all wells of the plate document by clicking the upper-left corner of the plate grid.
7. Using the tables below as a reference, verify that the peak for the spectrum of the pure dye occurs at the correct filter. For documentation, **Print** the spectra for each dye (**Alt + Print Scrn**; then open a new blank Microsoft Word document and **Ctrl V** to paste the spectra). If the peak for the spectra of a dye

# Unit Policy Manual

## Forensic Biology

occurs in the wrong filter, you may have run the wrong dye plate during the calibration. Repeat the procedure using the correct dye.

Instrument	Pure Dye	Filter	Peak (nm)	
7500 System	FAM	A	~520 nm	
	VIC	B	~550 nm	
	NED	C	~580 nm	
	ROX	D	~610 nm	

8. Select **File > Close**. **Important!** Do not close a plate document until you have extracted it. During the calibration, the software creates plate documents for each pure dye plate as it was run. You must extract each one individually before closing it.
9. Repeat steps 1 through 8 to extract the calibration data for the remaining pure dyes.

### 6.7.6 Running the TaqMan RNase P Plate Data on the ABI 7500 SDS

#### MATERIALS REQUIRED:

1. Applied Biosystems Real-Time PCR System Spectral Calibration Kit

# Unit Policy Manual

## Forensic Biology

2. TaqMan RNase P Instrument Verification Plate (Part No. 4350584)
3. Centrifuge and vortex

Purpose The TaqMan RNase P Instrument Verification Plate run verifies the performance of an Applied Biosystems 7500 SDS instrument. The RNase P plate is pre-loaded with the reagents necessary for the detection and quantification of genomic copies of the human RNase P gene.

The RNase P plate contains five replicate groups of standards, two unknown populations and four no template control wells. After the run, the SDS software will assess the instrument performance.

Preparing the TaqMan RNase P Plate Retrieve a prepared TaqMan RNase P Verification Plate kit from the freezer in the amplified DNA room and allow the plate to thaw (approximately 5 minutes). Vortex and centrifuge the thawed plate to ensure that the liquid is in the bottom of the plate wells. If the RNase P plate has a compression pad on it, remove it from the plate.

### Creating a Plate Document for the RNase P Verification Run

1. Start the instrument and put the RNase P plate in the instrument.
2. Open the SDS software and click **File > New**.
3. In the New Document Dialog box:
  - a. Select **Assay > Absolute Quantification (standard curve)**.
  - b. Select **Container > 96-Well Clear**.
  - c. Select **Template > AQ RNase P Install**
  - d. In the Operator field, enter a name for the RNase P plate (i.e. RNase P *MMDDYY*) click **Finish**
  - e. In the SDS software, select **File > Save As**. (Navigate to D\:>AppliedBiosystems>SDS Documents and Save)

Performing the Verification Run In the SDS software: Select the **Instrument** tab and click **Start**. (The instrument default parameters should be correct. The RNase P plate volume is 50ul). The instrument begins the run which lasts approximately 1.5 hours. After the run is complete, click **OK**.

### Analyzing the TaqMan RNase P Plate Data

1. Select **Analysis > Analysis Settings**. Select **Auto C<sub>t</sub>** >Click **OK**. Click the green arrow or select **Analyze**.
2. Assess the results. In the plate document: Select the **Results** tab then select the **Amplification Plot** tab.

# Unit Policy Manual

## Forensic Biology

4. Display the data: from the Amplification Plot tab:
  - a. Click the upper-left corner of the plate grid to select all wells.
  - b. Select **Data > C<sub>t</sub> vs. Well Position** to display the plots. Save this data.
5. Verify the uniformity of each replicate population by comparing the groupings of C<sub>t</sub> values.

If outliers are present, omit and reanalyze (*see page 93 of the 7500 Installation and Maintenance Guide for trouble shooting, this will apply to the 7000, as well*).
6. In the results tab, select the **Standard Curve** tab and verify that the R<sup>2</sup> value is  $\geq 0.990$ . Save and print this data.
7. In the **Report** tab, calculate the verification value of the 5,000-copy and 10,000-copy populations:
  - a. For the 5,000-copy populations, in the Report tab, scroll to a sample in the 5K population. Apply the value in the Mean Qty and StdDev Qty columns to the following equation:
$$5K \text{ value} = \text{Mean Qty} + 3(\text{StdDev Qty})$$
  - b. For the 10,000-copy populations, in the Report tab, scroll to a sample in the 10K population. Apply the value in the Mean Qty and StdDev Qty columns to the following equation:
$$10K \text{ value} = \text{Mean Qty} - 3(\text{StdDev Qty})$$
8. Compare the values of the 10K and 5K-copy populations. If the 10K value is greater than the 5K value the 7500 has passed.
9. With all wells chosen, print the Report. Save the Tabular Report Data and the Standard. You may discard the Raw Data and the Delta Rn vs Cycle Data pages. It is recommended that the calculations performed above be written on one of the Tabular Report Data pages.
10. Close Program. If asked to Save, click “yes”.

Remove the plate and discard. (note: the plate cannot be re-used)

### 6.7.7 Monitoring and Replacing the Lamp on the 7500 SDS Instrument

#### 6.7.7.1 Monitoring the Lamp Status

# Unit Policy Manual

## Forensic Biology

The halogen lamp located in the 7500 SDS Instrument has a life span of approximately 1,000 hours. If the lamp has exceeded 1,000 hours, or if a message is displayed that recommends changing the lamp, procedure 6.8.7.2 should be followed.

1. Select **File > New**.
2. In the New Document Wizard, click **Finish**.

In the SDS Software, select **Instrument>Lamp Status/Replacement**. In the Lamp Status/Replacement dialog box, the usage hours and lamp current are displayed. The Condition field will indicate one of the following:

Good—the lamp is functioning well. There is no need to replace the lamp bulb at this time. Click **Close**.

Failed—The lamp bulb must be replaced. Click **Close**, then replace the lamp.

Change Soon—the lamp bulb usage is above 2000 hours. It is recommended to replace the lamp soon. Click **Close**, then replace the lamp.

*Warning messages may be displayed before or during a run that indicate low lamp current. See page 121 of the Installation and Maintenance Guide for these messages, what they are describing, and how to proceed.*

### 6.7.7.2 Replacing the Halogen Lamp on the 7500 SDS Instrument

The halogen lamp located in the 7500 SDS Instrument has a life span of approximately 1,000 hours. If the lamp has exceeded 1,000 hours, or if a message is displayed that recommends changing the lamp, this procedure should be followed.

#### MATERIALS REQUIRED:

1. Halogen bulb (12V, 75W)
2. Small pointed object, for example a screwdriver.

**Important: Wear powder-free gloves when you handle the lamp.**

1. Power off, then unplug the 7500 SDS Instrument. Allow the instrument to cool for 15 minutes.
2. Open the access door to the Instrument:  
Insert a thin screwdriver or other object into the keyhole on the edge of the front access door, then push to unlatch the door. Open the door.

# Unit Policy Manual

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## Forensic Biology

3. Remove the lamp from the instrument:  
Slide the lamp release lever downward. Firmly grasp the lamp and lift it up and out of the slotted mount.
4. Inspect the lamp for signs of failure (see the Installation and Maintenance Guide page 124 for indications of lamp failure).
5. Place the new lamp into the instrument  
Slide the lamp release lever upward. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.
6. Close the access door.
7. Plug in and power on the 7500 SDS Instrument.
8. Open the 7500 System software program.  
If the Quick Startup document dialog box is not open, select **File** > **New**. In the New Document wizard, click **Finish**. In the SDS software, select **Instrument** > **Lamp Status/Replacement** and click on **Reset lamp timer** then close.
9. In the ROI Inspector dialog box, select **Lamp Control** > **Idle**.
10. While the instrument is running, look through the grating of the access door and verify that the lamp is illuminated, then click **Done**.

**Important!** After replacing the lamp, the following calibrations must be performed: ROI Calibration, Background and Optical Calibrations, Pure Dye Calibration, Instrument Performance Verification (RNase P Plate).

# Unit Policy Manual

## Forensic Biology

### 6.7.8 PROCEDURE FOR THE CALIBRATION CHECKS OF THE 9700 THERMOCYCLERS

The thermocyclers used for the polymerase chain reaction amplification of DNA are located in the Amplification Room of the Forensic Biology Unit. A calibration verification test and temperature non-uniformity test are conducted on each thermocycler approximately every six months. The verifications are conducted by an outside vendor.

## 7.0 COLLECTION KITS AND SUPPLY ORDERING

### 7.1 PREPARATION OF EVIDENCE COLLECTION KITS

The Forensic Biology Unit provides officers, investigators, and sexual assault nurses with six types of kits used in the collection of biological evidence. Currently, the City of San Diego has contracts with Children's Hospital Chadwick Center and Independent Forensic Services (IFS) for conducting sexual assault examinations and collecting evidence related to sexual assault cases. In addition to kits, these medical service providers may request supplies, such as extra swabs and paper bags, used in sexual assault exams. IFS and Children's Hospital will call when they need kits and supplies. The requested kits and supplies should be gathered and taken to the main laboratory clerical office for pick-up. Communications should be contacted at 685-0451 to request the delivery of the kits to IFS or Children's Hospital. Sub-stations and other investigative units of the Police Department will call for kits as needed. Often, an officer or investigator will come to the laboratory to pick up kits; other times kits will need to be brought to the Property Room and the property clerk will deliver them to the sub-stations.

In general, the Laboratory Technician is responsible for preparing and disseminating the kits, however, the unit as a whole is responsible for these kits. A point person may be assigned the responsibility of these kits. A minimum stock should be kept on hand at all times in order to fill requests promptly. Kit components should also be prepared and kept on hand. Kits and kit components should be made on a sterile surface. Use protective gloves and a laboratory coat when preparing and handling kits and kit components.

#### 7.1.1 REFERENCE MOUTH SWAB COLLECTION KITS

Keep 60-100 kits on hand at all times.

Place the following items in a Reference Mouth Swab Collection Kit envelope (a manila envelope with 'SDPD REFERENCE MOUTH SWAB COLLECTION KIT' printed on the front):

- 2 sterile, paper-wrapped swabs
- 1 small "SDPD Reference Mouth Swabs" envelope
- 1 smaller manila envelope
- 1 evidence seal



# Unit Policy Manual

## Forensic Biology

- 1 pair of large latex gloves
- 1 copy of 'Instructions for the Collection of Reference Mouth Swabs'
- 1 copy of the SDPD consent form

Individual kits can be packaged together in large paper bags containing 20 kits.

### 7.1.2 BIOLOGICAL STAIN COLLECTION KIT

Keep 60-100 kits on hand at all times.

Place the following items in a Biological Stain Collection Kit envelope (a manila envelope with 'BIOLOGICAL STAIN EVIDENCE COLLECTION KIT' printed on the front):

- 2 sterile, paper-wrapped swabs
- 1 tube containing sterile water
- 1 small 'SDPD BIOLOGICAL EVIDENCE SAMPLE' envelope
- 1 smaller manila envelope
- 1 evidence seal
- 1 pair of large latex gloves
- 1 copy of 'Instructions for the Collection of a Biological Stain'

### 7.1.3 VICTIM SEXUAL ASSAULT EXAMINATION KIT

Keep 60-80 kits on hand at all times.

Place the following items in a Victim San Diego Regional Sexual Assault Evidence Collection Kit envelope:

- 5 swube tubes
- 1 Pubic Hair Combing envelope with comb
- 1 Head Hair envelope with bindle
- 1 Left Fingernail Scrapings envelope with 2 fingernail swabs in a bindle
- 1 Right Fingernail Scrapings envelope with 2 fingernail swabs in a bindle
- 1 piece of tissue paper
- 1 evidence seal
- 1 victim label sheet (1 Cervical Swabs, 1 Vaginal Swabs, 1 External Genital Swabs, 1 Oral swabs, 1 Rectal Swabs sticker, 1 Left Breast Swabs, and 1 Right Breast Swabs sticker)

### 7.1.4 SUSPECT SEXUAL ASSAULT EXAMINATION KIT

Keep 40-60 kits on hand at all times.

Place the following items in a Suspect San Diego Regional Sexual Assault Evidence Collection kit envelope:

# Unit Policy Manual

## Forensic Biology

- 5 swube tubes
- 1 Pubic Hair Combing envelope with comb
- 1 Head Hair envelope with bindle
- 1 Pubic Hair envelope with bindle
- 1 Left Finger scrapings envelope with 2 fingernail swabs in a bindle
- 1 Right Finger scrapings envelope with 2 fingernail swabs in a bindle
- 1 piece of tissue paper
- 1 evidence seal
- 1 suspect label sheet (1 Penile Swabs, 1 Scrotal Swabs, 1 Oral swabs, 1 Left hand fingers swab, and 1 right hand fingers swab stickers)

### 7.1.5 PHYSIOLOGICAL SAMPLES KIT

Keep 60-80 kits on hand at all times.

Place the following items in a Physiological Samples San Diego Regional Sexual Assault Evidence Collection Kit envelope:

- 2 grey-capped blood vials (Vacutainer®) each in a cigar tube
- 1 urine bottle with sodium fluoride
- 1 custody seal
- 1 evidence seal

Blood vials and cigar tubes are provided by the Narcotics Unit.

### 7.1.6 FETAL TISSUE COLLECTION KIT

Prepared as needed.

Place the following items in a manila envelope:

- 2 blue-capped 50mL conical vials (1 is a spare)
- Evidence seals
- Procedure for collecting aborted fetal material

A cooler and ice pack will be provided to any investigators for transporting the fetal material to the Property Room. Reference mouth swab collection kits should also be provided to investigators for the purpose of collecting samples from the mother and alleged father.

## 7.2 ORDERING AND RECEIVING COMMERCIAL KITS

# Unit Policy Manual

## Forensic Biology

### 7.2.1 Ordering procedure

Requests for supplies, reagents, and DNA typing kits will be placed in the Supply Order Log (electronic file) located on the department network (G:\Laboratory\Tony\Ordering Log).

When orders are placed, the date ordered and the order confirmation number will be logged into the Supply Order Log.

### 7.2.2 Receiving supplies

The receipt of the supplies will be logged into the Supply Order Log. The packing slip received with the supplies is initialed and dated then submitted to the Administrative Aide in charge of purchasing for the laboratory.

### 7.2.3 Receiving chemicals

The receipt of the chemicals will be logged into the Supply Order Log. The packing slip received with the chemicals is initialed and dated then submitted to the Administrative Aide in charge of purchasing for the laboratory.

A record of the chemicals on hand in the Forensic Biology Unit will be maintained. Any new chemicals (i.e. not previously ordered/received) will require an MSDS to be on file in the Unit.

Chemicals need to have the date and the initials of the receiver on the container.

### 7.2.4 Receiving DNA kits

The kits used for DNA extraction, quantification, and typing must all be recorded in the Kit Receipt Log upon receipt. In general, the lot numbers of every component of the kit are recorded as well as the lot number of the kit. Kit components may be stored in different locations and, thus, all kits components must be labeled with the lot number of the whole kit, or alternatively with an abbreviated kit ID # (e.g. IDP#-YY).

#### 7.2.4.1 QUANTIFILER KITS

Record lot numbers of the PCR reaction mix, DNA standard, primer, and whole kit in the Kit Receipt Log. The PCR reaction mix should be labeled with the whole kit lot number and stored in the reagent refrigerator. The remainder of the kit (in the original box) will be stored in the reagent freezer. Place 'Not QC'd, Do Not Use' stickers on all kit components. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

#### 7.2.4.2 STR TYPING KITS

# Unit Policy Manual

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## Forensic Biology

Record lot numbers of all components of the kit and the whole kit lot number in the Kit Receipt Log. The PCR reagents box should be labeled with the whole kit lot number and stored in the reagent refrigerator. If AmpliTaq is part of the kit, it must be placed in a freezer holder with tube numbered (1 thru X), labeled with the whole kit lot number and stored in the reagent freezer. The ladder will be labeled with the whole kit lot number and stored in the reagent refrigerator in the Amplification Room. Place 'Not QC'd, Do Not Use' stickers on all kit components. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

### **7.2.4.3 QIAGEN DNA INVESTIGATOR KITS (EZ1 or QiaSymphony)**

Record lot numbers of all kit components, except the buffers and proteinase K, and the whole kit in the Kit Receipt Log. Discard the proteinase K and buffers. Place 'Not QC'd, Do Not Use' stickers on all kits until quality control testing has been completed. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

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