Unit Policy Manual

Forensic Biology

SAN DIEGO POLICE DEPARTMENT

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1. QUALITY ASSURANCE

1.1. UNIT DESCRIPTION

1.1.1. The Forensic Biology Unit must be staffed by a minimum of two of qualified analysts.

1.1.1.1. If the unit is staffed by fewer than two qualified analysts, the laboratory will seek to outsource requests for serology and DNA analysis until the unit is sufficiently staffed to resume issuing reports.

1.1.2. The unit must have a DNA Technical Manager (DNA Technical Leader) and a CODIS Administrator.

1.1.3. The unit structure and its place within the San Diego Police Department Crime Laboratory will be detailed in the Department organizational chart.

1.1.4. The Forensic Biology laboratory will be divided into three distinct analytical areas: a laboratory area which used for the purposes of screening items of evidence and the DNA extraction process; an area dedicated to PCR setup; and a separate area dedicated to the preparation and analysis of amplified DNA.

1.2. UNIT FUNCTIONS

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

1.2.2. The serological testing capabilities of the Forensic Biology Unit will include presumptive and (where possible) confirmatory tests for components of blood, semen, and saliva.

1.2.3. The DNA testing capabilities of the Forensic Biology Unit will include both autosomal and Y-chromosome STR DNA testing.

1.2.4. Any additional testing methods performed on forensic casework must be validated prior to use.

1.2.5. Additional duties performed by the Forensic Biology Unit include courtroom testimony, and crime scene reconstruction.

1.3. PERSONNEL AND JOB DESCRIPTIONS

1.3.1. SUPERVISING CRIMINALIST

The Supervising Criminalist(s) 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 of all assigned 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

Duties and responsibilities:

A. Responsible for the technical operation of the laboratory as outlined in the FBI Director's Quality Assurance Standards for DNA Testing Laboratories.

B. Authority to initiate, suspend, and resume technical operations for the laboratory or an individual.

C. Reviewing, revising and approving any DNA-related technical policy or procedure prior to final issuance by the Quality Manager.

D. Overseeing, reviewing and approving DNA method validation or modification, including completion of validation documentation, prior to final approval by Quality Manager.

E. Overseeing and approving training of DNA analysts, laboratory technicians, and technical reviewers in DNA analytical procedures, including completion of training documentation. This includes reviewing, verifying, and approving the academic transcripts for newly qualified analysts and technical reviewers.

F. Approving the technical specifications for outsourcing agreements. Review potential conflicts of interest when contract employees are employed by multiple NDIS participating and/or vendor laboratories.

G. Reviewing internal and external DNA Audit documents, and if applicable, approve any Corrective Action reports involving a DNA technical issue.

H. Reviewing the policies and procedures of the DNA Unit on an annual basis.

I. Reviewing and approving the training, quality assurance, and proficiency testing programs in the DNA Unit.

J. Acting as a technical reference for the Quality Manager.

K. Performing some technical reviews of DNA case files.

L. Acting as a mediator when necessary in the technical review of DNA casework in the Forensic Biology Unit.

M. Providing technical consultation as needed to the members of the Forensic Biology Unit.

N. Working with Supervising Criminalists and Quality Manager to ensure compliance of the DNA analysts with QA and unit policies and procedures.

O. Conducting casework analysis.

P. Preparing reports as required by the Crime Laboratory Manager and Assistant Crime Laboratory Managers.

1.3.3. CODIS ADMINISTRATOR

Duties and responsibilities:

A. Oversees the security and integrity of the local CODIS network.

B. Has authority to terminate an analyst's or the laboratory's participation in CODIS until the reliability and security of the computer data can be assured in the event an issue with the data is identified.

C. Oversees the entering of DNA profiles from casework into the local database.

D. Ensures that DNA profiles are searched appropriately against the CODIS databases.

E. Schedules and documents the CODIS computer training of casework analysts.

F. Ensures that the security of data stored in CODIS is in accordance with state and/or federal law and NDIS operational procedures.

G. Ensures that the quality of data stored in CODIS is in accordance with state and/or federal law and NDIS operational procedures.

H. Ensures that matches are dispositioned in accordance with NDIS operational procedures.

I. Oversees entering data into the CODIS hit tracking Crosslinks databases.

J. Ensures upgrades to the CODIS software are performed as required by NDIS.

K. Schedules and documents the yearly training of analysts in the operation of CODIS software.

L. Disseminates CODIS information as needed between laboratories and 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 covering the subjects of 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, and 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.

C. Conducts DNA analysis to determine possible associations between the evidence and any reference samples provided.

D. Writes reports summarizing the findings and provides 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 a Supervising Criminalist, Assistant Crime Laboratory Manager, or the Crime Laboratory Manager.

H. Informs the Supervising Criminalist or DNA Technical Manager of problems that develop at any stage of a case.

I. Uses the Forensic Biology Unit Technical Manual, Forensic Biology Unit Policy Manual, and Quality 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. Laboratory technicians may locate and identify biological material on items of physical evidence and sample items of evidence.

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 biological evidence collection kits and reference mouth swab kits are prepared for departmental use or for the collection of evidence at area hospitals.

G. Receives, inventories, screens, and preserves items of physical evidence.

H. Locates and identifies biological material.

I. Writes reports summarizing the findings and provides expert testimony in court.

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

1.3.6. INTERN/VOLUNTEERS

Interns (students) and volunteers (non-students) 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 guidance 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 after receiving the appropriate training and qualifying tests, and under the supervision of the DNA Technical Manager.

1.4. QAS VERSION FOR EDUCATION, EXPERIENCE, AND TRAINING REQUIREMENTS

1.4.1. The date of hire, promotion, or transfer of an employee into a classification in the Forensic Biology Unit which may include DNA analysis of evidence items (i.e. Criminalist 1 or above) will be used for the purposes of determining which version of the QAS will apply for evaluating the education, training, and experience requirements of analysts.

1.5. TRAINING PROGRAM

1.5.1. The training program information will be maintained in the Forensic Biology Training Program Manual.

1.5.1.1. Documentation of training program completion will be maintained by the Quality Manager.

1.5.1.2. Documentation of training and competency for new methods will be maintained by the Quality Manager.

1.5.2. Each analyst will be provided 8 hours (cumulative minimum) of documented training (i.e., continuing education) per year in subject areas relevant to DNA typing.

1.5.2.1. Documentation of completed continuing education will be maintained by the DNA Technical Manager.

1.5.2.2. Documentation of internal training will include:

1.5.2.2.1. Title of the training presentation/program;

1.5.2.2.2. A copy of the presentation/training provided;

1.5.2.2.3. Date of the training/presentation;

1.5.2.2.4. Attendance list;

1.5.2.2.5. Hours of training; and

1.5.2.2.6. If the presenter is external to the laboratory, the presenter's CV. (Note that trainers internal to the Crime Laboratory have their SOQs on file in PowerDMS).

1.5.2.3. Participation and completion of programs based on multimedia or internet delivery must be formally recorded and approved by the DNA Technical Manager.

1.6. LITERATURE REVIEW

1.6.1. Annual review of scientific literature will be documented within the laboratory's document management system (i.e., PowerDMS).

1.6.2. Analysts within the Forensic Biology Unit will be provided copies of relevant literature (at least one full article, abstracts, or miscellaneous publications) periodically throughout the year.

1.7. TECHNICAL LEAD CONTINGENCY PLAN

1.7.1. In the event of a vacancy in the DNA Technical Manager position, the Crime Laboratory Manager will appoint a qualified person as DNA Technical Leader on an interim basis until the City process for filling the vacant DNA Technical Manager position can be completed.

1.8. NON-CONFORMING WORK

1.8.1. Quality Incident Summary forms (FB network H:\Worksheets\QA Worksheets) will be completed by analysts for:

- any malfunction or problem with laboratory equipment,
- when control samples fail to provide the expected results,
- when a technical policy or procedure was violated in the process of analysis,
- when an analyst error compromises the analytical process of the evidence, or
- when a potential association is made to the elimination DNA database for a sample that is suitable for comparisons.

1.8.2. Quality Incident Summaries will address the following (if applicable):

1.8.2.1. Definition and evaluation of the non-conformance

1.8.2.2. Actions Taken to address the non-conformance

1.8.2.3. Root cause (if determined)

1.8.2.4. Reported results opinions or interpretations

1.8.3. Quality Incident Summaries will be submitted to the DNA Technical Manager, or designee, for further evaluation. If corrective actions are required, as defined in section 8.7 of the Quality Manual, the Quality Manager will be notified.

1.8.3.1. Non-conformances will be evaluated to determine its significance or impact on the current analysis.

1.8.3.2. Non-conformances will be evaluated to determine its significance or impact to previous analysis. The Quality Manager will be notified if previous work is deemed to be affected.

1.8.3.3. Appropriate actions will be taken to remediate non-conformances. Appropriate actions will be determined based on 1.5.3.1 and 1.5.3.2.

1.8.4. Quality Incident Summaries will be tracked to identify trends. If a trend is identified, the Quality Manager will be notified.

1.8.5. Non-conformances involving failed controls or potential associations with the elimination DNA database with likelihood ratio HPD values greater than 99 will be disclosed in the report.

1.9. PROFICIENCY TESTING

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

1.9.1. Each DNA analyst in the Forensic Biology Unit must complete two external DNA proficiency tests in each calendar year with an interval between the tests of no less than four months and no more than eight months.

1.9.2. The due date of the proficiency test (as listed by the provider) will be the date by which proficiency tests are tracked.

1.9.3. At least one proficiency test per year will involve body fluid identification.

1.9.4. Proficiency tests will be worked in the same manner as casework and undergo both technical and administrative review.

1.9.5. Autosomal STR testing must be run on each semiannual proficiency test.

1.9.6. YSTR testing (for those qualified) must be run on at least one proficiency test per calendar year. YSTR testing must be performed on each *sample* that has male DNA detected.

Note: If a sample containing male DNA has multiple fractions with male DNA present (i.e., sperm and non-sperm fractions), only one fraction must have YSTR testing performed on it.

1.9.7. Analysts must enter into the proficiency testing program within 8 months of completion of the training program.

1.9.8. The DNA Technical Manager will compare the results obtained by each analyst to the consensus results from the test provider, determine the outcome of the test, and inform analysts of the outcome.

1.9.9. Analysts will initial their proficiency test packets, acknowledging the outcome of the test.

1.9.10. Proficiency tests will be maintained by the Quality Manager.

1.10. AUDITS

1.10.1. Audits of the Forensic Biology Unit will be performed in accordance with the timelines identified in the FBI Director's *Quality Assurance Standards for DNA Testing Laboratories*.

1.10.2. Documentation of both internal and external audits of the Forensic Biology Unit will be retained indefinitely by the DNA Technical Manager.

1.11. 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).

1.11.1. ELECTRONIC DATA STORAGE

Data backup is performed automatically and is managed through the Data Systems Department of the San Diego Police Department. Please contact SDPD Data Systems for additional information regarding the management and security of the Forensic Biology computer network.

1.12. QUALITY CONTROL AND CRITICAL REAGENTS

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

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

1.12.2. SAMPLES AVAILABLE FOR QUALITY CONTROL TESTING

Only samples with known GlobalFiler 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.

1.12.3. QUALITY CONTROL TESTING OF CRITICAL REAGENTS

1.12.3.1. Before any critical reagent can be used for casework analysis it must undergo quality control testing.

1.12.3.2. Quality control analysis will undergo technical review and review by the DNA technical Manager, or designee.

1.12.3.3. The results of controls, NIST SRM, and known samples must be in complete agreement with their expected results order for a critical reagent to pass QC.

1.12.3.4. If the QC analysis does not pass, a second QC analysis will be used to confirm the original result.

1.12.3.4.1. If the second QC analysis demonstrates the reliability of the kit, it may be used for casework analysis.

1.12.3.4.2. If the second QC confirms a problem with the critical reagent, then the critical reagents tested will not be approved for casework and, if applicable, the vendor of the reagent will be contacted.

1.12.3.5. 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 DNA Investigator kits: At least five aliquots of a known volume of blood (liquid and/or stains) from one or more known samples will be extracted with the new lot of EZ1 Investigator kits. The same amounts/samples of blood will be extracted with a currently QC'ed lot of EZ1 Investigator Kits. The DNA amounts recovered from the samples purified with the new lot of kits should be similar to the DNA amounts recovered from the currently QC'ed lot of kits. At least two samples purified with the new lot of EZ1 Investigator Kits and several concentrated (note: concentration factor not to exceed 8x) reagent blanks (some eluted in TE and some eluted in water) 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 Trio kits: The DNA standard tubes from the new lot of Quantifiler Trio 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 single-source samples with known genotypes should also be quantified using the new Quantifiler Trio 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 Trio Kit. Successful typing of the known samples will serve to demonstrate the reliability of the new kit lot(s).

GlobalFiler DNA typing kits: 007 Control DNA should be defrosted, pooled, and re-distributed into the DNA standard tubes prior to performing the QC testing. Two known single-source samples will be typed along with the 007 Control 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 GlobalFiler. (See also the NIST Testing policy below)

Yfiler Plus DNA typing kits: New kit lots will be tested with two known single-source male 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.

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

1.13. NIST TESTING OF DNA PROCEDURES

NIST Standard Reference Material or NIST-traceable samples will be used when validating new quantification kits or DNA typing kits.

1.14. EQUIPMENT VERIFICATIONS

1.14.1. New critical equipment (1.12.2) will require validation, or verification prior to implementation into casework.

1.14.2. Verifications will be performed on the equipment that has been identified as critical to the analysis of samples in the Forensic Biology Unit. The equipment listed below will be assessed at the approximate intervals indicated. If any of the instruments in the list is taken out of service a verification will be performed prior to re-initiating casework on the instrument.

Equipment Item	Location	Schedule (approximate)
Pipettes	Forensic Biology Main Lab and Amplification Room	Every six months
Balances	Forensic Biology Main Lab and Amplification Room	Annually
Thermomixers	Forensic Biology Main Lab	Every six months
QIAcubes	Forensic Biology Main Lab	Annually
BioRobot EZ1s BioRobot EZ1 Advanced XL Ez2 Connect Fx Volume Test	Forensic Biology Main Lab	Every six months
Hamilton Nimbus	Forensic Biology PCR Setup	Every six months

Equipment Item	Location	Schedule (approximate)
QuantStudio 5	Amplification Room	
Background calibration		Every six months
ROI/Uniformity		Every two years
Dye Calibration		Every two years
RNAse P		Every two years
Thermal cyclers	Amplification Room	Every six months
Genetic Analyzers	Amplification Room	Annually
QIAgility	Amplification Room	Annually

See the equipment maintenance section for additional information on equipment care.

1.14.3. Pipettes will be verified by an external service provider based on the pipette manufacturer's systematic (accuracy) and random (precision) error tolerances.

1.14.4. **VERIFICATIONS**

If any maintenance, repair, or service is conducted on a piece of equipment, a performance verification will be performed.

1.14.4.1. **QIAcubes** - preventive maintenance will be performed on the instrument on a yearly basis. After any service visit, including the yearly preventive maintenance, the QIAcube(s) will be evaluated with a performance verification. This verification will aim to establish if the instrument performance is acceptable for casework applications. This is accomplished by determining if the service issues were corrected and that the instrument(s) can still perform the methods as expected. One way of determining this is to run a set of mixed sperm/non-sperm cell samples through the QIAcube and continue the analysis through both quantification, amplification, and CE analysis. Satisfactory performance will be determined by the ability to separate the sperm and non-sperm fractions of the verification samples.

1.14.4.2. **BioRobot EZ1, BioRobot EZ1 Advanced XL, and EZ2 Connect Fx** every six months a volume test will be conducted on all BioRobot EZ1, BioRobot EZ1 Advanced XL, and EZ2 Connect Fx instruments to determine whether pipetting performance is still optimal. See the equipment maintenance protocols section (section 6) for additional information. A passing result must be within 5% of the pipetted volume for all channels within the instrument.

1.14.4.3. **Hamilton Nimbus** – preventive maintenance will be performed on the Nimbus instruments approximately every 6 months. As part of the preventive maintenance visit, the pipetting accuracy and precision are checked for all 4 channels. This is documented in the volume verification report.

After any service visit, including the yearly preventive maintenance, the Nimbus instruments will be evaluated with a performance verification. This verification will aim to establish if the instrument performance is acceptable for casework applications. This is accomplished by determining if the service issues were corrected and that the instrument(s) can still perform the methods as expected. One way of determining this is to run a set of samples through both quantification setup and amplification setup protocols. Replicate samples may be used to assess pipetting volume accuracy and precision during the quantification setup.

Any changes to the methods (i.e., robotic instructions underlying the procedure) will require a modification study to determine whether the changes have adversely affected performance.

1.14.4.4. **QuantStudio 5** – preventative maintenance will be performed on the QuantStudio 5 every year. This maintenance includes temperature verification of the thermal cycler block.

One way of verifying the QuantStudio 5 after service visits in which temperature verification is not included, is by

quantifying two single-source samples with known genotypes. 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.

1.14.4.5. **Thermal Cyclers** – preventative maintenance will be performed on the thermal cyclers every 6 months. This maintenance includes temperature verification of the thermal cycler block.

One way of verifying the thermal cyclers after service visits in which temperature verification is not included is by amplifying and typing two single-source samples with known genotypes, the 007 Control DNA positive control, and an amplification blank 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.

1.14.4.6. **Genetic Analyzers** - the QC analysis of critical reagents will serve as the annual performance verification 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.

After any service visit, including the yearly preventive maintenance, genetic analyzers will be evaluated with a performance verification. This verification will aim to establish if the instrument performance is acceptable for casework applications. This is accomplished by determining if the service issues were corrected (if applicable), electrophoresis capabilities (e.g., resolution) are acceptable, and that the sensitivity of the instrument has not been

altered substantially. One way of determining this would be to evaluate data from a set of samples run pre-service and comparing it to data from the same samples run post-service.

1.14.4.7. QIAgility –One way of verifying the QIAgility after service visits is to perform an evaluation of the instruments pipetting against manual pipetting of the same samples (using the same volumes for manual as on the QIAgility). This will be accomplished based on the QIAgility pipetting of a known set of replicate samples for analysis on the genetic analyzer. The results of the replicate QIAgility pipetted samples will be compared against the same replicate samples prepared manually. Total detected peak heights of both sets of samples will be used to measure the performance of the QIAgility. Although several variables will affect the ultimate peak heights on the 3500, ideally the difference between the total peak heights both sets of samples should be less than 10%.

1.15. 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.

1.16. MICROSCOPES

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

1.17. QUALITY ASSURANCE DOCUMENTATION IN THE FORENSIC BIOLOGY UNIT

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

Log Name	Location
Kit Receipt Log	Electronic
Reagent Preparation Logs	Electronic
Supply Order Log	Electronic
DNA Extraction	Electronic
QIAGEN Robot Reagent QC	Electronic
GlobalFiler QC	Electronic
Y-STR QC	Electronic
Quantifiler Trio QC	Electronic
Unexpected Results	Electronic
FB Incident Log	Electronic
Staff Associations	Electronic
Balance Calibration Tests (Quarterly)	Electronic
Pipette Calibration	Electronic
Nimbus Maintenance	Electronic
Thermal Cycler Calibration & Uniformity	Electronic
3500 Maintenance	Electronic
Ovens & Fridges Temperature Log	Electronic
QIAGEN EZ1, EZ1, etc. Volume Test	Electronic
QIAcube Maintenance	Electronic

2. TECHNICAL RECORDS

2.1. REPORT FORMAT

The Biology Unit will use the standardized report format located in the Quality Manual. Where available, CII numbers will be included in the report.

2.1.1. BACKGROUND SECTION

2.1.1.1. A background section of the report will only be used for:

2.1.1.1.1. Describing corrections to, and referencing, original reports in Report Amendments; or,

2.1.1.1.2. Referencing external data used to render conclusions in the report.

2.1.1.1.3. Documenting unusual circumstances with DNA Technical Manager and/or Supervising Criminalist approval.

2.1.2. CASE-TO-CASE HIT NOTIFICATIONS

2.1.2.1. Will be issued for any new associations between evidence items in different cases, or to individuals in multiple cases.

2.1.2.2. Are required when any SDPD cases have been associated through the database to:

2.1.2.2.1. Any other SDPD case;

2.1.2.2.2. Cases from an outside agency;

2.1.2.2.3. A named individual.

2.1.2.3. Do not require technical review, but must be administratively reviewed prior to issuance.

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. TECHNICAL RECORDS

2.2.1. The technical record assembled by the analyst will contain the original of the final signed report, the work request(s), any communications or documentation directly relevant to testing, and the complete analytical record (or a reference as to where the data are located).

2.2.1.1. When testing information from a separate case is relied upon for interpretations within a technical record (e.g., a reference sample worked as part of a different case), a pink worksheet indicating where the information originated will be included as a numbered page of the technical record.

2.2.1.2. This pink sheet will serve to notify clerical that additional material is required in the event of a discovery request.

2.2.1.3. Documentation that does not directly pertain to the technical record (i.e., that does not direct testing, indicate or specify the items to be tested, or is not related to testing activities performed within the analytical record (see below) will not be included as part of the numbered pages of the record.

2.2.2. The technical record will subject to both technical and administrative reviews.

2.2.3. **ANALYTICAL RECORD**

2.2.3.1. The analytical record will contain the following (as applicable):

2.2.3.1.1. Pink sheet indicating the case #(s) for any information relied upon not part of the current analysis (see 2.2.1.1 and 2.2.1.2)

2.2.3.1.2. Request for analysis

2.2.3.1.3. Documentation of task-relevant communications

2.2.3.1.4. Evidence inventory and analysis notes, including any serology tests results

2.2.3.1.5. DNA extraction, quantification, amplification, and capillary electrophoresis notes (or a reference to where that information may be found)

2.2.3.1.6. DNA results (electropherograms)

2.2.3.1.7. Interpretation information/worksheets

2.2.3.1.8. STRmix results page with diagnostic information of the MCMC

2.2.3.1.9. Statistical evaluations

2.2.3.1.10. H_dTT evaluations for any association deemed investigatively relevant

2.2.3.1.11. CODIS worksheet printouts for any samples to be uploaded to CODIS

2.2.3.1.12. Details of any staff or CODIS database matches

2.2.3.1.13. Disposition information for derivative evidence and work product

2.2.4. CASEWORK BATCHING

2.2.4.1. Analysts may group the analytical records for multiple cases together into a single analytical record called an analytical batch.

2.2.4.2. Each case in the casework batch must receive its own individual report.

2.2.4.3. Technical and administrative reviews will be conducted on analytical batches.

2.2.4.4. Completed analytical batches must be stored electronically on the FB network such that they are available when the technical records that rely on them are reviewed.

2.2.5. NOTETAKING

2.2.5.1. Notes will be taken contemporaneously with examinations or analysis.

2.2.5.2. The analyst will take notes with the expectation that they, a technical reviewer, or other person with foundational knowledge will be able to reconstruct what was done in terms of examinations conducted, results obtained, testing completed, and any sampling.

2.2.5.3. Page numbers and analyst initials will appear on each page of the technical record.

2.2.5.4. Technical record pages will reflect the date upon which the laboratory activities were conducted.

Note: Emails, electropherograms, and STRmix report pages that contain dates do not require additional dates on them unless notations are made after the initial printing/interpretation of the data.

2.2.5.5. 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 the discovery process for unrelated cases.

2.2.5.6. The condition of evidence packaging will be noted (e.g., damaged, unsealed, etc.) when evidence is retrieved from the Property Room or Laboratory Vault.

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

2.2.5.8. 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.

2.2.5.9. Any assumptions used will be documented in the technical record. The determination of the number of contributors must be on the electropherogram, and, for mixtures, the locus/loci used to determine the number of contributors must also be included.

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:

Please refer to the Autosomal STR Interpretation Guidelines (or Y-STR Interpretation Guidelines section) section of the Forensic Biology Unit 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 Manual.

2.4.1. ANALYICAL BATCH REVIEW

2.4.1.1. The following list will guide analysts in providing a complete technical review of the analytical worksheets for a batch of samples. The review of an analytical batch is designed to streamline the review process by not duplicating data review.

Extraction Worksheets
Appropriate volume of Digest Buffer/ Prot. K (and DTT)
Correct Preparation date for Digest Buffer/ Prot. K (and DTT)
Incubation time ≥ required minimum
Appropriate elution volume
TE or dH2O Elution box checked
EZ1/EZ2 instrument indicated for each sample
Correct Reagent Strip Lot #
Reagent blanks included
EZ1/EZ2 or QIAcube instrument and protocol indicated
Quantitation Worksheets
Correct Quantifiler Trio lot #
QS5 Instrument listed
Setup listed
Master Mix Preparation was accurately calculated
Calibrators are within acceptable range
TE Amp Blank quantification is *undetermined* or 0.0000ng/uL
All reagent blanks quantification are *undetermined* or 0.0000ng/uL
Amplification Worksheets
Correct Amplification Kit (GlobalFiler or Yfiler Plus) lot #
Correct TE Prep Date
Veriti Instrument listed
Setup listed
Master Mix Preparation was accurately calculated
Correct volume for Positive Control (007) added
Correct volume for TE Amp Blank added
If samples combined, corresponding RBs are combined
Correct volume for Reagent Blanks added
Dilutions (and/or) concentration procedures listed below table

3500 Sample Setup Worksheets
Formamide Blank and Formamide ISS included
Two ladders included
Positive and negative control included
Reagent blanks included
3500 Instrument listed
Sample setup listed
Correct Reagent Lot #s (Formamide, ISS, Ladder)
GeneMapper Analysis (electronically viewed)
Sample page included in packet
At least 1 evidence and 1 reference ladder passed (if applicable)
Correct Sample Type selected
Correct Analysis Method and Panel selected for Evidence and Reference
All GS600 – Appropriate peak heights and resolution
Positive Control passed (correct types obtained, any additional peaks explainable)
All Reagent Blanks (No detected DNA types)
TE Amp Blank (No detected DNA types)
If increased injections were used, controls also injected appropriately
Administrative Review
Date, initials, page numbers and analytical batch name on each page
Corrections are made in the appropriate format
Analyst has corrected/ resolved all concerns raised by the tech reviewer
Worksheets are complete
Analytical batch technical review worksheet is complete

2.4.2. TECHNICAL REVIEW

2.4.2.1. Each technical record in the Forensic Biology Unit will have a technical review conducted on it by a second qualified analyst.

2.4.2.2. Technical review distribution within the unit will be at the discretion of management.

2.4.2.3. Technical review will ensure the accuracy of the final report and that established Forensic Biology protocols and policies are being applied to case analysis.

2.4.2.4. Technical review will ensure that the conclusions in the report are supported by the data in the analytical record (i.e., case notes, sketches, photos, etc.).

2.4.2.5. If changes are made as a result of the technical review that affect the opinions and interpretations in the report, the authorization date must be updated to reflect the date of those changes.

2.4.2.6. Technical review will be completed in a timely manner (typically within 14 calendar days).

2.4.2.6.1. Any delays that will cause this time period to be exceeded must be communicated with the supervisor so that alternative arrangements may be made, if necessary.

2.4.2.6.2. Any requirements for rush reviews must be communicated with a supervisor so it can be assigned for priority review directly.

2.4.2.7. Unresolved differences between the case analyst and the reviewing analyst will be mediated by the DNA Technical Manager.

2.4.2.8. The date of the completion of the technical review will be documented in the technical record per the crime laboratory Quality Manual.

2.4.2.9. The following list must be used to guide analysts in providing a complete technical review. 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 based upon documented policies and procedures.

2.4.2.9.1. Technical review worksheets will not be maintained in the technical record.

Notes
DNA request has supervisor (or designee) initials
References - justification for collection documented and/or consent form included
All evidence and reference samples approved by supervisor (or designee) for analysis were tested
Permission to consume evidence obtained (if applicable)
Communications concerning the case are recorded
Notes/worksheets are complete and legible
Case file has pink case reference sheet
Evidence Sampling
Written/photographic description of packaging, evidence examined, & samples collected included
Photographs contain a scale
Approved methods used to locate and identify biological material
Controls used for presumptive tests
Documentation that evidence was labeled, repackaged, and sealed
Analytical Notes
Completed Analytical Batch (AB) review worksheet included
Analytical notes not part of analytical batch contain info required per AB checklist
QIS documentation present in notes or in AB
Appropriate template amount amplified for samples. Appropriate discontinuation policies followed
Electropherograms
Electropherograms present for all relevant samples
Multiple injections were performed where necessary. If so, results generally consistent/expected.
Artifacts are labeled and analyst-derived allele designations accurate
Unresolved peaks are documented with allele designation, size, and height

Interpretation
Reasoning regarding number of contributors is documented on electropherogram
Correct STRmix profiling kit/injection time was used
Conclusions based on visual comparison are documented
Evidence (.txt) files used in MCMC accurate and complete, including unresolved peaks
Comparisons made to all relevant reference samples and reference (.txt) files accurate and complete
MCMC conditioned on reference(s) if supported by scenario and data/LR
MCMC genotypes, weights, and mixture proportions meet qualitative expectations (if not, informed priors used)
Sex determining loci checked, interpreted, and documented
STRmix database search results evaluated using Caucasian DB
H_dTT (using all populations) run for MCMC(s) with probative associations
No large LR disparities between loci (e.g. inclusionary LRs at all but one locus of 0)
For Y-STRs, designation of major and minor contributors is appropriate
For Y-STRs, appropriate comparisons were performed and conclusions drawn
For Y-STRs, appropriate statistical assessment of evidence performed
CODIS
Requests for elimination samples documented
CODIS sheets included for applicable and eligible evidence and suspect profiles
Samples conditioned on suspect references or forensic unknowns to refine CODIS searches (if applicable)
MME is appropriate for level of CODIS being searched, risk probability is ≥ 40%
CODIS sheets are complete and correct, including specimen category and DNA types
For CODIS hits, LRs have been performed to assess "goodness-of-fit".

Report
Header is correct on all report pages
Amended report designation and appropriate background included
Evidence Examined & Opinions/Interpretations sections have correct barcodes & item descriptions
Evidence Examined contains results of serology testing (& test details listed under table)
Evidence Examined contains correct discontinuation code(s) and appendix reference statement
DNA typing kit(s) listed
Opinions/Interpretations list number of contributors and mixture proportions
Opinions/Interpretations reflect conclusions made in notes (i.e. inclusions/exclusions and verbal wording, all comparisons listed, CODIS database search statements)
Reported LR for associations is the lowest HPD of all four populations
Sub-sub source LR reported for inclusions with $>10^6$-fold difference between LR and HPD
CODIS search level is indicated for each component
Signature block is correct and complete
Analytical batch(es) listed in report
Information from QIS documented appropriately

2.4.2.10. TECHNICAL REVIEW EXPECTATIONS

2.4.2.10.1. Corrections to the notes regarding spelling or grammar can be made by the technical reviewer.

2.4.2.10.2. Any differences of opinion that cannot be clarified during the initial back-and-forth of a review will be discussed in person.

2.4.2.10.3. Continued disagreements will be mediated by the DNA Technical Manager.

2.4.2.10.4. Continued disagreements will involve the Quality Manager or Supervising Criminalist.

2.4.2.10.5. Analysts involved in the discussion may elect to have further discussions with the Crime Laboratory Manager concerning the technical issue.

2.4.3. **ADMINISTRATIVE REVIEW**

2.4.3.1. The Quality Manual details the basic elements of the administrative review.

2.4.3.2. The administrative review will be documented per the Quality Manual.

2.4.3.3. The total number of pages notation in the technical record can be completed by the administrative reviewer as part of the review process.

2.4.3.4. Administrative reviewers will be responsible for completing the portions of SARTonQ related to the completion and issuing of the report.

2.4.3.5. The following list will be used as a guide to provide 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.

2.4.3.5.1. Administrative worksheets will not be maintained in the technical record.

Notes
Date, analyst initials and case/incident number on each page
Corrections are made in the appropriate format
All technical review comments/corrections have been addressed
Every page is numbered, in order, and total number of pages indicated on 1st page
CODIS sheets have been copied for upload

Report
Proper grammar, spelling, and Style Guide format
Evidence Examined section contains all agreed upon items from request
Disposition section included
Analyst signature and reviewer initials are in place
Report appendix included
Other
Update evidence databases as appropriate (i.e., EvidenceOnQ, SARTonQ)
Chain of custody for all items is complete
Sub-item designations added to comments section in EvidenceOnQ

3. EVIDENCE POLICIES

3.1. The Forensic Biology Unit will accept case submissions from all investigative units within the Department. Case submissions will be handled as described in the Quality Manual.

3.2. Any reference DNA sample requested must have a consent form submitted in order to have the sample analyzed, unless it has been collected: from a suspect that is a felony arrestee; by court order; from a suspect who is a 4th waiver subject; or from sexual assault victim as part of the SAFE kit collection.

3.3. Consent forms will be maintained as a numbered page within the technical record.

3.4. Analysts will adhere to the consumption of evidence policy in the Quality Manual.

3.4.1. 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 consume 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.

3.5. SEXUAL ASSAULT EVIDENCE SAMPLING POLICIES

3.5.1. VICTIM SEXUAL ASSAULT EVIDENCE KIT SAMPLING POLICIES

3.5.1.1. Half of the evidence swabs selected for testing from a victim SAFE kit (also referred to as SART kits) will be consumed for initial testing (e.g., one of two swabs collected or half of a single swab). The remaining half of any evidence may be sampled (if needed - see section 3.10.5.4.1 in the discontinuation policy section) in accordance with the laboratory's consumption of evidence policies. Microscope slides are required to be prepared for sexual assault evidence that is being consumed for testing and is suspected of containing semen (see section 3.6.15).

3.5.1.2. A total of two samples from each general body area will be tested if present in the kit. These areas, based on the Cal OES Standard, are the interior vaginal (if applicable), external genital area, anal area, oral area, and breast/pectoral area.

3.5.1.2.1. Victims under the age of 14 or victims with a specified intellectual disability (i.e., specified in lab request)

3.5.1.2.1.1. All swabs collected from the five body areas mentioned in 3.5.1.2 will be sampled for testing.

3.5.1.2.1.2. Swabs from within and immediately surrounding body cavities (i.e. perioral, perianal, and external genital swabs) will be sampled for differential extraction.

3.5.1.2.1.3. Body surface swabs will be sampled for non-differential extraction with DTT

3.5.1.2.1.4. If the medical report or DNA request specifically indicates external ejaculation on an alternate body area (e.g., stomach), then the swab from that area will also be sampled for differential extraction.

3.5.1.2.2. Kits from Female Victims, Aged 14 and Over

3.5.1.2.2.1. The cervical, vaginal, external genital, oral, peri-oral, anal, and peri-anal areas (if present in the kit) will be sampled for differential extraction. Note: if more than two sets of samples are listed from a body area (e.g., multiple external genital swab present in kit – vestibular and vulvar swabs), only two sets of samples from that area will be tested.

3.5.1.2.2.2. The mons pubis, right breast, and left breast areas (if present in the kit) will be sampled for non-differential extraction with DTT.

3.5.1.2.2.3. If the medical report specifically indicates external ejaculation on an alternate body area (e.g., stomach), then the swab from that area will also be sampled for differential extraction.

3.5.1.2.3. Kits from Male Victims, Aged 14 and Over

3.5.1.2.3.1. The penile, scrotal, oral, peri-oral, anal, and peri-anal areas (if present in the kit) will be sampled for differential extraction.

3.5.1.2.3.2. The right and left breast/pectoral areas (if present in the kit) will be sampled for non-differential extraction with DTT.

3.5.1.2.3.3. If the medical report specifically indicates external ejaculation on an alternate body area (e.g., stomach), then the swab from that area will also be sampled for differential extraction.

3.5.1.3. If the medical report indicates a condom was worn, sampling of the SAFE kit will proceed based on 3.5.1.2. Condoms will be analyzed pursuant to separate requests submitted/accepted by the laboratory.

3.5.1.4. Any initial medical report-based sampling of evidence outside of the above policies requires supervisor approval.

3.5.1.5. Investigators will be informed via the DNA report if untested samples are present in the SAFE kit that can be analyzed. Any subsequent analysis performed on these samples will be pursuant to separate requests submitted/accepted by the laboratory.

3.5.1.6. Testing of SAFE kit samples will be done in accordance with the discontinuation of analysis policies.

3.5.2. SUSPECT SEXUAL ASSAULT EVIDENCE KIT SAMPLING POLICIES

3.5.2.1. Suspect sexual assault forensic evidence kits will be sampled based on the circumstances of the case or based on the testing needs of the investigation.

3.5.2.2. Half of the evidence swabs selected for testing from a suspect SAFE kit (also referred to as SART kits) will be consumed for initial testing (e.g., one of two swabs collected or half of a single swab). The remaining half of any evidence may be sampled (if needed - see section 3.10.5.4.1 in the discontinuation policy section) in accordance with the laboratory's consumption of evidence policies. Microscope slides are required to be prepared for sexual assault evidence that is being consumed for testing and is suspected of containing semen (see section 3.6.15).

3.5.3. SAMPLING POLICIES FOR OTHER EVIDENCE SUSPECTED OF CONTAINING SEMEN

3.5.3.1. If evidence other than a swab from a sexual assault evidence kit is suspected to contain semen based upon provided scenario information and/or serological screening results, a differential extraction will be performed.

3.5.3.1.1. If the serological results are negative for the test(s) for semen or if a previous differential extraction of the sample suggested that semen was not present, a non-differential extraction with DTT or a general DNA extraction may be performed.

3.5.3.2. Microscope slides are required to be prepared for sexual assault evidence that is being consumed for testing and is suspected of containing semen (see section 3.6.15).

3.5.3.2.1. Consumption in this context refers to the entirety of: a suspected stain (or collection of stains in proximity), a swab/swatch used to collect an entire suspected semen stain, or a swab/swatch used to collect a suspected semen stain from an item/area that cannot be reswabbed.

3.6. **FACILITIES AND EVIDENCE CONTROL:** See the laboratory's Quality Manual for lab-wide policies regarding facilities and evidence control. The following are additional unit policies:

3.6.1. Separate areas are designated within the Unit for evidence evaluation and DNA extraction, PCR setup, and for the amplification and analysis of amplified DNA. Procedures will be performed in the appropriate area for that analysis.

3.6.2. Pipettes used in post-amplification processes must not be used in the pre-amplification areas without thorough decontamination and cleaning.

3.6.3. The door of the room containing amplified DNA will remain closed except for passage.

3.6.4. Amplified DNA will only be stored in the refrigerators and freezers in the post-amplification area.

3.6.5. No reagents used in the post amplification processes will be stored in any refrigerator or freezer in the pre-amplification areas.

3.6.6. Evidence and reagents should be stored separately in their designated locations while in the Forensic Biology Unit.

3.6.7. Records will be maintained in the Reagent Preparation Log for any reagent prepared for casework application. The record will indicate the preparer, the date of preparation, and the lot numbers, or date of preparation, of components used in the preparation of the new reagent.

3.6.8. Any visitors to the lab areas within Forensic Biology will be required to wear a mask and gloves during their time spent in pre-amplification lab areas. Visitors to lab areas will be asked to provide a sample for the elimination database.

3.6.9. Analysts must wear appropriate personal protective equipment when examining evidence. This includes, at a minimum, a lab coat and gloves. Facemasks are recommended.

3.6.10. Prior to analysis, work areas must be cleaned with a 10-20% bleach solution. The work area will be covered with clean paper or bench mats prior to evidence analysis. 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.

3.6.11. After analysis, items will be returned to the original packaging. 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. If the item cannot be returned to the original packaging, new packaging can be created, but the original packaging must be packaged with the item.

3.6.12. Analysts must avoid compromising any information written on the evidence packaging. Notation will be made in the case notes if the contents of the packaging could not be accessed without breaking an existing evidence seal.

3.6.13. Evidence collected from the suspect(s) must not be sampled or extracted at the same time as evidence collected from the victim. Case scenario information relating to the locations evidence items were collected must be considered so that evidence collected from locations associated with the victim are not extracted with evidence from locations associated with the suspect. The DNA extraction of evidence and reference samples must be separated by time and/or place.

3.6.14. Hair evidence must be photographed if consuming more than half the length of a hair for DNA analysis. Close-up (i.e., macro-lens photography) is recommended.

3.6.15. The creation of microscope slides is mandatory for sexual assault evidence suspected of containing semen any time a sample/stain will be consumed for DNA testing.

3.6.15.1. If the sample being consumed has previously had a microscope slide prepared from the evidence, and is available for examination, there is no need to create a new microscope slide.

3.6.16. Slides prepared will be dispositioned either with extracted DNA work product, or added to the packaging (e.g., SAFE kit) from which the microscopically examined item originally came from.

3.6.17. Derivative evidence generated as a result of analysis will be assigned a new barcode in FileOnQ system, a notation as to final disposition will be made in the notes and report.

3.6.18. If an evidence item is swabbed for DNA analysis, a notation will be made within the FileOnQ evidence tracking database regarding any additional associated barcodes created.

3.6.19. Scissors used for sampling evidence must be cleaned thoroughly with bleach, soap and water, alcohol, or by flaming between sampling different stains. Alternatively, a fresh scalpel blade will be used for each evidence sample.

3.6.20. 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.

3.6.21. All reagents and solutions will be autoclaved where possible. Sterile water and sterile plastics or glassware will be used for solutions that cannot be autoclaved.

3.6.22. Reagents made in-house shall be labeled with the identity of the reagent, the date of preparation and/or expiration, and the identity of the individual preparing the reagent.

3.6.23. Reagents will be stored as small aliquots to minimize the effects of any contamination events.

3.6.24. The lot(s) of reagents used for each set of analyses will be recorded.

3.6.25. 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 or removing liquid from evidence sample tubes.

3.6.26. The DNA extraction/purification of questioned samples will be performed separately from the DNA extraction/purification of reference 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.

3.6.27. 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. 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 consumed during a first analysis.

3.6.28. A reagent blank will be subjected to all of the reagents and manipulations of the DNA analysis process as evidence samples.

3.6.28.1. Reagent blanks will receive the same volume of reagents as the highest volume used for evidence samples.

3.6.28.2. Reagents blanks will undergo the same manipulations as the most manipulated evidence sample associated with it.

3.6.28.3. At least one reagent blank will be carried through amplification and analysis with its associated samples.

3.6.28.3.1. Any reagent blank that has DNA detected (above 0.0000ng/uL) at quantification will be amplified and analyzed.

3.6.28.3.2. If multiple reagents blanks are associated with an extraction set and a single reagent blank has DNA detected in it (above 0.0000ng/uL), if no other amplification kit is likely to be used, then at least two reagent blanks should be amplified (i.e., the one with DNA detected and one without DNA detected) in an attempt to identify the detected DNA as an isolated event.

3.6.28.3.3. If multiple reagents blanks associated with an extraction set have DNA (above 0.0000ng/uL) in them, the DNA Technical Manager must be consulted on the most appropriate course of action. That consultation will be documented in the technical record.

3.6.28.4. Reagent blanks do not require microscopic examination, nor have any volume spotted onto a microscope slide when associated samples are being examined microscopically.

3.6.29. When performing manual PCR setup, DNA must be added to the tubes after all reagents have been added. This practice reduces the possibility of inadvertent transfer of DNA between samples and kit reagents.

3.6.30. Only one sample tube will be open at a time during DNA addition in manual PCR setups.

3.6.31. Only disposable lab coats are to be used in the post-amplification area. Always remove gloves and lab coats before exiting the post-amplification area.

3.6.32. All surfaces in the Amplification Area should be cleaned with 10-20% bleach solution before and after setting up samples for analysis.

3.6.33. All equipment found in the Amplification Area is dedicated to that area and will never be removed unless proper decontamination procedures have been employed.

3.6.34. DNA interpretations must be documented in the technical record.

3.6.35. CODIS sheets for evidence samples must be part of the technical record.

3.7. DNA SAMPLE STORAGE

3.7.1. Short term storage of extracted DNA will be the dedicated freezer in the PCR setup area. DNA extracts should be stored in closed containers or sealed bags.

3.7.2. Long-term storage of extracted DNA will be in the Property Room. A new barcode number must be created for extracted DNA samples. All new items in FileOnQ require an incident number to be assigned. Any new incident number assigned to extracted DNA samples will follow the format of: "ExtDNA*analystthreeletterinitials*MMDDYY".

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

3.7.3.1. 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.

3.8. POLICIES REGARDING FREEZER PACKETS

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

3.8.2. Freezer packets will be marked with the incident or case number and the barcode(s) of the evidence contained within.

3.8.3. 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 FileOnQ under the appropriate incident number.

3.8.4. Documentation of the newly generated items should be included in the case notes and report.

3.9. UNIQUE IDENTIFIERS

3.9.1. The barcode number will uniquely identify each item stored in the Property Room or Laboratory Vault under the FileOnQ system.

3.9.2. Items stored under the old system will be uniquely identified by the case number (or lab sequence number), property tag number, and item number.

3.9.3. Multiple items contained within a single package will be inventoried.

3.9.3.1. The contents of a SAFE kit will all be given sub-item designations, which will be noted in the barcode system for that item.

3.9.3.2. When multiple items are contained within a single package, the items analyzed will be given sub-item designations, which will be noted in the barcode system for the package.

3.9.3.3. If items are not being analyzed, but are being noted for inventory purpose only, similar items may be grouped together (e.g. miscellaneous papers) rather than individually described.

3.9.4. Any stains, cuttings, or swabs (i.e., derivative evidence) taken from items must be able to be tracked back to the location from which they were sampled.

3.9.5. Derivative evidence that will be impounded into the Property Room will require the generation of a new item in the barcode system under the appropriate incident number. This new barcode will uniquely identify these pieces of derivative evidence.

3.9.6. All items included on a laboratory request to be examined by the laboratory (excluding internally produced requests) are considered evidence.

3.9.7. All cell pellets, extracted DNA, and amplified DNA will be considered the work product of the DNA laboratory.

3.9.8. 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.

3.10. DISCONTINUING ANALYSIS OF SAMPLES

3.10.1. Variables, such as the existence of other probative samples, or the case scenario may affect the decision to discontinue the analysis of a sample.

3.10.2. Quantification information provides information into the likelihood of obtaining interpretable DNA results, and the decision to discontinue analysis will be made after quantification of the sample(s).

3.10.3. If a sample contains insufficient DNA to reasonably obtain an interpretable DNA profile, it will be discontinued.

3.10.3.1. Samples that are expected to have a low amount of DNA will have 8µL of extract quantified.

3.10.3.2. If 8µL of a sample are quantified AND the amount of DNA in the extract is ≤0.0005ng/µL for all three targets (small autosomal, large autosomal, and male), and all controls indicate the quantification has performed as expected, the samples are unlikely to yield interpretable DNA results with the GlobalFiler amplification kit and the sample will be discontinued.

3.10.3.2.1. Analysts will consult with the DNA Technical Manager if they believe the control results cause them to doubt the reliability of the data from the quantification assay.

3.10.3.2.2. Documented permission from the DNA Technical Manager is required to amplify samples for which quantification values ≤0.0005ng/µL are obtained.

3.10.3.2.3. For samples extracted with an associated hair shaft control or substrate sample:

3.10.3.2.3.1. If the *evidence sample* associated with a hair shaft control or substrate sample is being amplified, the hair shaft control or substrate sample should be amplified even if the amount of DNA in the extract from the hair shaft control or substrate sample is ≤0.0005ng/µL for all three targets.

3.10.3.2.3.2. If analysis of the evidence sample associated with the hair shaft control or substrate sample is discontinued, analysis of the hair shaft control or substrate sample will be discontinued.

3.10.4. In cases where multiple samples from the same item are collected for the same purpose (e.g., DNA sample collected from two different areas on a shirt for wearer information), if one sample has sufficient DNA additional samples collected for the same purpose may be discontinued.

3.10.5. For sexual assault cases:

3.10.5.1. Analysts should determine from the scenario whether all human DNA (male and female) from a sample is investigatively relevant or whether only the male portion of the DNA is investigatively relevant.

3.10.5.2. For cases in which the victim is under the age of 14, has loss of awareness (as specified in medical report), or a

mental disability (specified in request); cases with multiple perpetrators; or single perpetrator cases in which the victim had prior sexual activity within 5 days:

3.10.5.2.1. For samples in which all human DNA is investigatively relevant, all samples with DNA (human or male) above the discontinuation threshold (>0.0005ng/µL) will be amplified.

3.10.5.2.2. For samples in which only male DNA is investigatively relevant, all samples with male DNA above the discontinuation threshold (>0.0005ng/µL) and with a human:male ratio below 75:1, will be amplified. See 3.10.5.4 for further discontinuation guidance.

For differentially extracted samples:

3.10.5.2.3. If only the sperm fraction is investigatively relevant and being typed, the associated non-sperm fraction can be discontinued. For many items, such as some clothing or bedding, there may be uncertainty in the history and/or use of the item, which makes the non-sperm fraction investigatively relevant to determine the wearer/user.

3.10.5.2.4. If the non-sperm fraction is investigatively relevant and being typed and the sperm fraction has >0.0005ng/µL male DNA detected with a human: male ratio below 75:1, both the sperm and non-sperm fractions must be DNA typed.

3.10.5.2.5. If the non-sperm fraction is investigatively relevant and the sperm-fraction has insufficient male DNA detected, only the non-sperm fraction will be DNA typed.

3.10.5.3. For cases with a single perpetrator scenario in which the victim is 14 years or older and has had no other sexual activity within 5 days:

3.10.5.3.1. For samples in which all human DNA is investigatively relevant, only one sample with DNA (human or male) above the discontinuation threshold (>0.0005ng/µL) from each body location tested (i.e. interior vaginal (if applicable), external genital area, anal area, oral area, and breast/pectoral area) will be amplified.

3.10.5.3.2. For samples in which only male DNA is investigatively relevant, only one sample with male DNA above the discontinuation threshold (>0.0005ng/µL) and with a human:male ratio below 75:1 from each body location tested (i.e. interior vaginal (if applicable), external genital area, anal area, oral area, and breast/pectoral area) will be amplified. See 3.10.5.4 for further discontinuation guidance.

For differentially extracted samples:

3.10.5.3.3. If only the sperm fraction is investigatively relevant and being typed, the associated non-sperm fraction can be discontinued. For many items, such as some clothing or bedding, there may be uncertainty in the history and/or use of the item, which makes the non-sperm fraction investigatively relevant to determine the wearer/user.

3.10.5.3.4. If the non-sperm fraction is investigatively relevant and being typed and the sperm fraction has >0.0005ng/µL male DNA detected with a human: male ratio below 75:1, both the sperm and non-sperm fractions must be DNA typed.

3.10.5.3.5. If the non-sperm fraction is investigatively relevant and the sperm-fraction has insufficient male DNA detected, only the non-sperm fraction will be DNA typed.

3.10.5.4. If only male DNA is investigatively relevant and samples/fractions have an insufficient amount of male DNA (≤0.0005ng/µL male DNA detected and/or human:male ratio

greater ≥75:1) the analysis of the samples/fractions will be discontinued unless they meet one of the criteria outlined below:

3.10.5.4.1. If all tested samples/fractions meet the requirements for discontinuation, but at least one sample has a low amount of male DNA detected (≤0.0005ng/µL) with a good human:male ratio (below 75:1) and has additional portion(s) of the sample that could be extracted, the analyst will select the single sample with remaining portion(s) of evidence which has the highest amount of male DNA and will seek and document supervisor permission to attempt to combine the sample with the remaining evidence in an attempt to obtain an interpretable DNA profile. If permission is obtained, testing of the remaining swab will proceed based on the laboratory consumption of evidence policy.

3.10.5.4.2. In the situation where sufficient male DNA is detected (>0.0005ng/µL male DNA detected), but the human:male ratio is greater than 75:1, YSTR testing may be considered if a suspect reference sample is available and no other fractions from the same body location or from the same item in the case are being typed.

3.10.5.4.3. When Y-STR analysis is possible, but not performed (e.g., because no suspect reference is available or because samples from the other body areas were tested for autosomal STR testing), the report will indicate that Y-STR testing can be performed upon request and the submission of a suspect reference sample.

3.10.6. If all of the evidence samples associated with a victim, witness, consensual partner, etc., have been discontinued under sections 3.10.3-3.10.5, analysis of the associated reference sample may be discontinued. If all of the associated evidence samples have been discontinued, any previously untested suspect reference samples should be tested for possible entry into CODIS, if applicable (see section 4.3).

3.11. USING EXTERNALLY-DERIVED DATA FOR LIKELIHOOD RATIO COMPARISONS

3.11.1. For the purpose of the policies in section 3.11, "externally-derived data" refers to reference DNA profiles, single source evidence profiles, or complete genotypes from partial DNA profiles generated in other accredited (e.g., ANAB or A2LA) DNA laboratories.

3.11.2. Externally-derived data must have been interpreted by the other laboratory in order to be used for comparisons.

3.11.3. The externally-derived data that will be used in comparisons are only that which represents complete genotypes at any locus.

3.11.4. The policies in 3.11 also apply to "goodness-of-fit" comparisons for CODIS hits.

4. 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, Legal, Unidentified Human Remains, Missing Persons, and Relatives of Missing Persons.

4.1. SDPD CODIS OPERATIONS

4.1.1. SDPD CODIS Operations are overseen by the CODIS Administrator. The CODIS Administrator will be supported by another qualified DNA analyst (Alternate CODIS Administrator). The CODIS Administrator and Alternate CODIS Administrator(s) comprise the CODIS Team.

4.1.1.1. The Alternate CODIS Administrator will perform CODIS duties as needed.

4.1.2. If the CODIS Administrator determines at any point that the CODIS network, software, or data have been compromised, the DNA Technical Manager will be immediately notified.

4.1.3. 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.

4.2. ENTRY OF DNA PROFILES INTO CODIS

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

4.2.2. DNA profiles selected for entry into CODIS will be documented in the technical record on worksheets referred to as CODIS sheets. If multiple items from the same case have the same DNA profile, only one CODIS sheet with the most discriminating data (i.e., rarest MME) should be used.

4.2.2.1. For cases with suspect samples submitted, evidence samples will be conditioned on the suspect reference DNA profile(s) to refine CODIS searches if:

a) The LRs associating the suspect(s) to the evidence is greater than 10^4; and
b) The evidence samples contain additional unknown components (other than the component associated to the suspect(s)).

4.2.2.2. For sex crimes, child abuse, or homicide with a suspected sexual element with unidentified Forensic Unknown (i.e., single source) data generated, LRs will be calculated for all evidence samples using the Forensic Unknown data in H1. Evidence samples will be conditioned on the Forensic Unknown DNA profile(s) to refine CODIS searches if the calculated LRs are greater than 10^4 if the evidence samples contain additional unknown components (other than the component associated to the Forensic Unknown). Components of evidence samples associated to the Forensic Unknown profile will not be searched in CODIS.

4.2.2.3. Conditioning on the suspect reference DNA profile(s) as described in section 4.2.2.1 and running an LR and/or conditioning on Forensic Unknown profiles as described in section 4.2.2.2 to refine CODIS searches is not required under the following scenarios:

a) If the genotype weights for the contributor associated with the applicable suspect reference or the Forensic Unknown profile are >99.5% at each locus.
b) Following consultation with a member of the CODIS Team, it is determined that conditioning on these profile(s) will not substantially alter the searchability of the additional contributors.

c) The evidence profile was previously analyzed and the reference sample profile(s) and/or Forensic Unknown profile(s) were generated as a part of supplemental analysis for comparison to the previous analysis.

4.2.3. DNA analysts shall perform keyboard searches of any unidentified, NDIS-eligible profiles prior to submitting CODIS sheets for those profiles.

4.2.4. Any consultations with the CODIS Team that affect or alter a potential CODIS search will be documented in the technical record.

4.2.5. CODIS sheets must be technically reviewed to ensure accuracy of the DNA types and specimen category, and acceptability for CODIS entry.

4.2.6. Any material changes made to the CODIS entry as a result of technical review must be documented.

4.2.7. Any material changes made by the CODIS Team after technical review is complete must be discussed with case analyst and documented within the analytical record.

4.2.8. Mixture and partial DNA records submitted to SDIS or NDIS will be reviewed to ensure the DNA records satisfy the statistical thresholds for match rarity set by the applicable SDIS or NDIS authorities.

4.2.9. Samples with alleles trimmed by COSTaR to meet the statistical thresholds for match rarity are calculated in COSTaR to determine how much the trimming effects the risk that a contributor's alleles are not included in the search. Samples below the 40% risk threshold should not be searched in CODIS and the CODIS sheets should not be included in the analytical record.

4.2.10. If a contributor to a sample is eligible for CODIS and has a single genotype at each autosomal locus with a genotype weight ≥99.5%, the contributor should be considered as a forensic unknown and

the CODIS sheet should be modified to reflect the single genotype at each locus.

4.2.11. 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.

4.2.12. DNA profiles derived from firearms in felon in possession cases are not eligible to be searched in CODIS.

4.2.13. DNA profiles or portions of DNA mixtures attributable solely to victims or elimination reference samples (boyfriend, husband, etc.) will not be entered into CODIS.

4.2.14. Forensic profiles in CODIS that are ultimately linked to consensual partners will be removed from the database.

4.2.15. CODIS sheets will be filled out for qualifying suspect samples (see section 4.3) that were not matched to a Forensic Unknown profile. If a suspect sample is associated to a Forensic Unknown sample, only a CODIS sheet for the Forensic Unknown should be filled out, indicating that it was associated to the suspect sample.

4.2.16. In general, CODIS sheets will be removed from the case file during the administrative review and given to the CODIS Team for entry. Copies of CODIS sheets must be retained within the technical record as numbered pages to document the CODIS search.

4.2.17. CODIS profiles will be reviewed for eligibility, correct DNA types, and appropriate specimen category prior to *upload* to local CODIS or beyond. This policy is not meant to prevent a keyboard search of DNA profiles in the local CODIS database.

4.2.18. For any profile uploaded into the CODIS databases, the level of CODIS searched will be disclosed in the report per ANAB and Quality Manual requirements. If a profile that is searchable per NDIS or State policies is not searched (e.g., due to similarity with another profile being searched), the reason for not searching the profile must be documented in the technical record.

4.2.18.1. If there is strong and/or very strong statistical support for the inclusion of a suspect to multiple items in a case, a single CODIS search of the evidence items/components associated with the suspect will be performed. The CODIS search with the highest MME/MRE should be selected for this search.

4.3. UPLOADING SUSPECT DNA PROFILES

4.3.1. DNA profiles from *adult* suspect samples analyzed by the SDPD laboratory will be uploaded to the local CODIS database if one of the following conditions have been met.

4.3.1.1. They have been arrested for a felony,

4.3.1.2. They are subject to conditions associated with fourth waiver status,

4.3.1.3. They are mandated by a court order to provide a DNA sample, or

4.3.1.4. They have provided consent to the collection of their DNA by signing a SDPD Consent Form acknowledging their DNA profile will be entered into the local CODIS database.

4.3.2. Suspect DNA profiles will be maintained indefinitely within the local CODIS database.

4.3.3. After a period of two years, suspect DNA profiles previously uploaded to the State and/or National CODIS database will be removed from the State and/or National level of CODIS and remain at the local level after the profile has been in State CODIS for a period of two years.

4.3.4. If an individual is eliminated as a suspect or can no longer be verified that they are a suspect in the case for which their Suspect DNA profile was submitted, the Suspect DNA profile from that individual will be removed from the all levels of the CODIS Database.

4.3.5. No Suspect DNA profiles obtained from juveniles (i.e., under the age of 18 at the time of collection) will be stored in the local CODIS database.

4.4. DATABASE SEARCHES AND MATCHES

4.4.1. Searches of the SDPD's local DNA database can occur prior to technical review and are also performed at the time of the entry of the profile into CODIS.

4.4.2. Newly generated DNA profiles for searching will be 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.

4.4.3. Information contained within the SDPD's local DNA database (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.

4.4.3.1. A "batch file" containing the information within LDIS will be provided for the purpose of comparing DNA profile information between jurisdictions.

4.4.3.2. When the SDPD Crime Laboratory receives a batch file from another agency, the batch file will be used for a search against LDIS.

4.4.3.3. DNA data from the external agency will not be stored with the SDPD's LDIS and ownership of the data will be retained by the agency from which it originated.

4.4.3.4. It will be communicated to outside agencies receiving batch files of DNA database data information from the SDPD LDIS that they must not store SDPD DNA data in their LDIS.

4.4.4. Dispositioning of potential hits within CODIS will be performed by the CODIS Administrator, or designee, in accordance with NDIS and SDIS procedures.

4.4.5. Potential hits at any level of CODIS will be evaluated for "goodness of fit". If the potential hit is deemed to be good, the notification process will be allowed to proceed. "Goodness of fit" of any potential database hit will be dependent on the specimen category.

4.4.5.1. Potential hits between forensic unknowns and any single source, non-partial specimen categories (i.e. forensic unknown, Convicted Offender, Legal, or Arrestee) can be assessed visually for goodness-of-fit.

4.4.5.1.1. If a potential hit is between an unsolved forensic unknown and a forensic unknown that has been associated to an individual who is a juvenile and/or whose suspect DNA profile was removed from local CODIS because they could no longer be verified to be a suspect, the analyst will report the possible association and request a new reference sample for comparison. Likelihood ratios for comparisons to the individual should not be reported

4.4.5.1.2. If a potential hit is between a forensic unknown and a profile in the local suspect category without a complete GlobalFiler dataset, the analyst should verify that the sample was collected pursuant to a 4th waiver, felony arrest, or court order.

4.4.5.1.2.1. If the sample was collected pursuant to one of these conditions, the sample should be retested with GlobalFiler to provide a complete profile for comparison and should be entered into LDIS under the current case number.

4.4.5.1.2.2. If this information cannot be verified, the analyst will not retest the reference sample, but will report the possible association and request a new reference sample for comparison.

4.4.5.2. Potential hits between a mixture and/or partial profile and any sample in a single source sample index will be assessed through calculation of a likelihood ratio.

Note: The likelihood ratio will assess the probability of the evidence given the DNA profile from the putative match is a component of the mixed or partial DNA profile searched against the probability of the evidence given the DNA profile from the putative match is not in the mixed or partial DNA profile searched.

4.4.5.2.1. If a potential hit is between an unsolved sample and a sample that has been associated to an individual who is a juvenile and/or whose suspect DNA profile was removed from local CODIS because they could no longer be verified to be a suspect, the analyst will report the possible association and request a new reference sample for comparison. Likelihood ratios for comparisons to the individual should not be reported

4.4.5.3. If a potential hit is between a mixture and/or partial DNA profile and a profile in the local suspect specimen category, the local suspect profile must have the full complement of data for the likelihood ratio comparison being reported (e.g., if the local suspect only has data at 15 loci, the reference sample must be tested for the full GlobalFiler dataset). Local suspect profiles without the complete GlobalFiler matching to a mixture or partial DNA profile should be retested with GlobalFiler, if possible.

4.4.5.3.1. The analyst should verify that the sample was collected pursuant to a 4th waiver, felony arrest, or court order.

4.4.5.3.1.1. If the sample was collected pursuant to one of these conditions, the sample should be entered into LDIS under the current case number.

4.4.5.3.1.2. If this information cannot be verified, and if the match is determined to be a forensic hit, the analyst will report the possible association and request a new reference sample for comparison.

4.4.5.4. Mixture and partial DNA profiles have ambiguity at one or more loci. Potential hits between two samples of these types require that the profiles be assessed to determine whether either profile has sufficient loci with a single, deduced genotype to allow for an LR calculation (e.g. a mixture with a single genotype deduced at seven or more loci). If at least one of the mixed and/or partial DNA profiles appears suitable, a subset of the profile comprising the loci with one deduced genotype will be used for the LR calculation.

4.4.5.5. In general, the likelihood ratio results used for the assessment will be the lowest 99% 1-sided HPD value for the four population groups.

4.4.5.5.1. Potential associations to the database with likelihood ratio results greater than fifty times the size of the relevant database in these comparisons will be declared as offender or forensic hits.

4.4.5.5.2. Potential associations with State and National databases will be evaluated by the CODIS Administrator, or designee. Additional data may be requested from the jurisdiction with which the putative hit has occurred to further evaluate its validity.

4.4.5.5.3. Likelihood ratio results between ten and fifty times the size of the relevant database will be further evaluated to determine if the putative associations will be reported.

4.4.5.5.3.1. This may be accomplished by assessing the electropherogram of the specimen and running any additional likelihood ratios (e.g., using a compound proposition) or MCMCs (e.g., conditioned) that would aid in determining if the hit could be a true association.

4.4.5.5.3.2. The CODIS Team will confer with the Technical Manager to discuss the potential hit.

4.4.5.5.3.3. If the findings indicate that the hit is not an association, the potential hit will be dispositioned as "No Match", and no further action will be required.

4.4.5.5.3.4. If the findings indicate the hit is a potential valid association, the compiled evaluation data will be technically reviewed by a qualified analyst and retained electronically in the case file folder. The disposition will be set as appropriate based on NDIS guidelines.

4.4.5.5.3.5. The process after any association has been deemed a hit will depend on the circumstances as well as the level of CODIS to which the hit was obtained:

4.4.5.5.3.5.1. If the sample hits to an evidence sample that has a state Offender or Arrestee hit already, and the reference sample from the named individual has not been tested by the SDPD laboratory, a Crosslinks report will be issued to the investigator and DNA analyst. The Crosslinks report will contain the approved 'Potential Hit' language.

4.4.5.5.3.5.2. If the sample hits to an evidence sample for which there is an association to a named individual, and that individual's sample has been tested by the SDPD laboratory, the investigator will be notified via email or a supplemental report will be issued using the approved 'Potential Hit' email language in section 4.4.5.4.3.6.

4.4.5.5.3.6. Hits to unsolved forensic sample(s) at LDIS, SDIS, or NDIS: A case-to-case association notification will be issued indicating no offender has been identified. This notification will be sent to the investigator and DNA analyst using the approved 'Potential Hit' language (below).

4.4.5.5.3.6.1. Potential Hit Email Template for possible hits between 10X and 50X of the relevant database size when an Offender has been identified.

Dear Investigator,

Attached please find a letter detailing an association between a sample in a case you may be working, and an offender in the CODIS database. This hit* may provide information regarding the investigation.

**Please note that this hit did not meet the statistical threshold used at the SDPD to establish confidence in the association of the named individual to the evidence sample. This potential database hit was subjected to further evaluation by the CODIS Team and DNA Technical Manager. The results of this evaluation indicate that this hit likely represents a valid association, and the name of the individual is being released.*

If this hit will be used in criminal prosecution of the case, a new reference sample must be collected from the identified

offender for analysis by the SDPD Crime Laboratory. This is mandated by guidelines from the California Department of Justice.

The sample identifier referenced in the attached is the case number and generally either the item number or the last three digits of the barcode number. If you have any questions concerning the evidence, please contact the analyst assigned to the case, copied on this email.

If a reference sample will not be submitted, please email Coral Luce at clluce@pd.sandiego.gov to indicate the status of this case and the reason for not submitting a reference sample. Examples include:

- Evidence is insufficient to pursue charges
- Evidence is not probative to solving crime
- District attorney's office declines to prosecute
- Victim is uncooperative or declines to prosecute
- Statute of limitations has passed for criminal charge

4.4.5.5.3.6.2. Potential Hit Email Template for possible hits between 10X and 50X of the relevant database size when an Offender has *not* been identified.

Dear Investigator,

Attached please find a notification detailing an association between a sample in a case you may be working, and a DNA sample from another case. This hit* may provide information regarding your investigation.

**Please note that this hit did not meet the statistical threshold used at the SDPD for establishing confidence in the association between the evidence samples. This potential hit was subjected to additional evaluation by the CODIS Team and DNA Technical Manager. The results of this evaluation indicate that this hit likely represents a valid association between the samples.*

At this time, an offender associated with the DNA evidence for the above listed cases has not been identified.

4.4.5.5.4. Likelihood ratio results used to assess forensic and offender hits will be retained in the case record, or electronically (G:\Misc DATA\CODIS\CODIS Matches).

4.4.5.5.5. If the database LR results are sufficiently high (e.g., $1.0x10^{11}$ or greater), it may not be necessary to perform an LR with HPD calculation as the value considering the HPD is unlikely to be less than the likelihood ratio threshold.

4.4.6. Information regarding forensic and offender hits must be included in at least one report or notification. Prior to a match report being written, investigators may be contacted regarding DNA hits. Information must be communicated accurately. The communication of a database hit must be documented in a communication log. Prior to releasing database hit information to an investigator, the results must be reviewed by a second analyst and the review documented in the notes.

4.4.7. When likelihood ratios are calculated comparing any suspect profile from the local CODIS database to any mixture or a partial evidence profiles, the suspect profile must have all the loci contained in the evidence sample. This requirement applies even if the suspect reference was associated to a Forensic Unknown (i.e., single source) component of a mixture.

4.5. MAINTENANCE OF AN ELIMINATION DATABASE IN CODIS

Section 3.0 in the Quality Manual addresses this database. The following are the policies for the collection and analysis of these samples:

4.5.1. The Quality Manager will coordinate sample collection of reference mouth swabs from the donors and coding of the sample.

4.5.2. Mouth swab samples from donors to the elimination database will only be retained with permission from the donor.

4.5.3. Elimination database samples will be analyzed by a qualified DNA analyst following the protocol used to analyze reference mouth swabs in casework.

4.5.4. The DNA Technical Manager, CODIS Manager, or other qualified analyst as designated, will review the analysis prior to entering the DNA profiles in any databases used for comparisons to evidence. The review will evaluate the following:

4.5.4.1. Approved protocols were followed for analysis of samples;

4.5.4.2. Sample data are entered into the Staff Index of the local CODIS database;

4.5.4.3. Sample data are added to STRmix elimination database;

4.5.4.4. Sample data are added to STRmix reference database for LR comparisons;

4.5.4.5. Sample data in all three locations are double checked for accuracy; and

4.5.4.6. Updated STRmix files are tested for functionality.

4.5.5. Electropherograms will be maintained for each sample.

4.6. REPORTING DNA RESULTS ASSOCIATED TO THE ELIMINATION DATABASE.

4.6.1. When an evidence DNA profile is found to match or potentially be associated with a profile in the elimination DNA database, the following will be done:

4.6.1.1. The CODIS match detail report, first page of the STRmix Database Search report, or the DBSearchResults text file

indicating the association will be included in the analytical record.

4.6.1.2. If the DNA profile in the case was found to match (or the mixture potentially includes) DNA from any elimination profile at an HPD LR value above 2 (i.e., limited support for inclusion or above), a Quality Incident Summary form will be completed detailing the association.

4.6.1.3. The Quality Incident Summary form will be submitted to the DNA Technical Manager, or designee. Any associations with HPD values above 10,000 will be routed through the Quality Manager.

4.6.1.4. Associations to the elimination database when the HPD LR is greater than 99 will be reflected in the report.

4.6.1.4.1. If the HPD LR value is greater than moderate support for inclusion (i.e., LR>10^4), the analyst will condition the evidence profile on the elimination sample, unless the elimination sample represents the sole unattributed contributor in the evidence sample.

4.6.1.4.2. The report will list the elimination sample as an assumed contributor if the profile is used as a conditioning profile, otherwise the LR and associated verbal scale descriptor will be reported.

4.6.1.4.3. If the LR value is in the moderate support for inclusion (i.e., 10^2<LR<10^4), the report will list the elimination sample as having moderate support for inclusion.

4.7. 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 laboratory 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.
- An on-site visit to the vendor laboratory is required.

4.7.1. **OWNERSHIP OF OUTSOURCED DATA**

4.7.1.1. SDPD ownership of outsourced data occurs when:

4.7.1.1.1. The SDPD will use any samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing (e.g., a vendor laboratory prepares an extract that will be analyzed by the SDPD laboratory);

4.7.1.1.2. The SDPD will interpret the data generated by the vendor laboratory;

4.7.1.1.3. The SDPD laboratory will issue a report on the results of the analysis; or

4.7.1.1.4. The SDPD will enter or search a DNA profile in CODIS from data generated by the vendor laboratory.

4.7.1.2. The SDPD will not take ownership of any cases deemed "negative". Negative cases are those that:

4.7.1.2.1. Are negative during initial screening;

4.7.1.2.2. Are determined to have quantities of DNA unsuitable for autosomal STR analysis; or

4.7.1.2.3. Yield DNA profiles consistent with originating from the reasonably expected donor of the item, consensual partners, or witnesses.

4.7.2. ON-SITE VISITS TO VENDOR LABORATORIES

4.7.2.1. Prior to the initiation of testing in an outsourcing agreement, an on-site visit of the vendor laboratory must be documented.

4.7.2.1.1. The on-site visit of the vendor laboratory must be conducted by the DNA Technical Manager, or another qualified (or previously qualified) analyst designated by the DNA Technical Manager, or Supervising Criminalist.

4.7.2.1.2. An on-site visit performed by the technical leader, or designated employee of an NDIS laboratory that uses the same technology, platform, and typing amplification test kit may be accepted by the DNA Technical Manager in lieu of performing the on-site visit outlined in 4.7.2.1.1.

4.7.2.2. An on-site visit to a vendor laboratory will include at a minimum:

4.7.2.2.1. An evaluation of the laboratory facilities to ensure the integrity of the evidence will be maintained while in the custody of the vendor lab.

4.7.2.2.2. 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.

4.7.2.2.3. An evaluation of any Corrective Actions taken by the laboratory in the previous six months prior to the on-site visit.

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

4.7.2.3. The on-site visit will be documented and maintained by the DNA Technical Manager.

4.7.2.4. If an outsourcing agreement with a vendor lab is extended beyond one year, annual on-site visits to the vendor laboratory must be documented.

4.7.2.4.1. As described in 4.7.2.1.2, an on-site visit conducted by another NDIS participating laboratory, or FBI designated employee may be accepted by the DNA Technical Manager.

4.7.3. QUALITY CONTROL OF OUTSOURCING DATA

4.7.3.1. Complete technical review will be conducted on approximately 3% of "negative cases" as defined in 4.7.1.2.

4.7.3.2. The SDPD Laboratory will conduct retesting on approximately 3% of all outsourced cases to ensure the accuracy of the testing results reported by the vendor laboratory. If a case selected for retesting had a searchable DNA profile obtained, but retesting would require the consumption of the original evidence, then a new case will be selected for retesting so as not to consume the original evidence. It is permissible to consume original evidence for any negative cases selected for retesting.

4.7.4. OUTSOURCED DATA

4.7.4.1. A technical review of the outsourced data will be performed prior to uploading any DNA profiles to SDIS (or NDIS).

4.7.4.2. The technical review of the outsourced data will be performed by an analyst:

4.7.4.2.1. That is, or was previously, qualified in the technology, platform, and typing amplification kit used to generate the data;

4.7.4.2.2. That participates in the SDPD external proficiency test program to the full extent they participate in casework and the review of outsourced data;

4.7.4.3. The technical review of the outsourced data will include a review of:

4.7.4.3.1. All DNA types to verify that they are supported by the raw and/or analyzed data;

4.7.4.3.2. All associated controls, internal lane standard and allelic ladders to verify the expected results were obtained;

4.7.4.3.3. The final report to verify the results and conclusions are supported by the data, and that each item tested has been addressed.

4.7.4.3.4. The DNA types, eligibility, and correct specimen category of any profile to be uploaded into CODIS.

4.7.4.4. A notification will be issued by the Forensic Biology Unit for any outsourced case in which a DNA profile is being searched in CODIS. The report will indicate the extent of the database search (i.e., local, State, or National).

4.7.4.5. If a profile from an outsourced case hits in CODIS to any sample for which the SDPD lab has analyzed the reference sample, the following notification will be sent to the appropriate investigative unit:

Investigator,

An association was obtained between a sample in "Outsourced Case # Specimen ID" and a sample in the CODIS database (see attached notification letter – if available).

This individual's reference sample has been analyzed by the SDPD DNA Unit.

If this association is relevant to the investigation and you would like a comparison between this reference profile data and the evidence, please contact Supervising Criminalist Shawn Montpetit (smontpetit@pd.sandiego.gov) and the DNA data from the reference sample can be provided to the vendor laboratory.

4.8. REINTERPRETATION OF LEGACY AMPLIFICATION KIT DATA

This policy is intended for situations when an analyst is required to *reinterpret* evidence sample data generated with legacy amplification test kits for comparisons to single source DNA profiles. This policy is not intended for situations when the original interpretation of evidence sample data generated with legacy test kits is not being modified (the data are not being reinterpreted).

Reinterpretation of legacy data may occur in accordance with the following:

1. If an analyst is currently qualified in an amplification test kit (trained and proficiency tested), and the analyst was previously qualified (trained and proficiency tested) in the legacy amplification test kit, the analyst can reinterpret the legacy data if the analyst has been proficiency tested on the legacy test kit within the last two calendar years. If an analyst has not been proficiency tested on a legacy test kit within the last two calendar years, then the DNA Technical Manager must document and approve the completion of the analyst's review of the validation data and standard operating procedures of the legacy test kit. If an analyst, who has completed the requirements to interpret legacy amplification test kit data, has not reviewed the required documents within the last two calendar

years, the DNA Technical Manager must document and approve the completion of the analyst's additional review.

2. If an analyst is currently qualified in an amplification test kit (trained and proficiency tested), but has never been previously qualified in the legacy amplification test kit, the analyst can reinterpret the legacy amplification test kit data if the analyst is trained in the legacy test kit interpretation protocols by a previously qualified analyst. The review of the validation data and standard operating procedures of the legacy test kit, training by the previously qualified analyst, and interpretation competency test must be completed by the analyst and documented and approved by the DNA Technical Manager. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

3. If a previously qualified analyst is unavailable to train a currently qualified analyst on a legacy amplification test kit, it is highly recommended that the current analyst does not reinterpret legacy data. However, if the current analyst must perform legacy interpretation without training from a previously qualified analyst, then it is recommended that the currently qualified analyst and DNA Technical Manager train in the legacy test kit interpretation protocols, review validation data and standard operating procedures. The training and interpretation competency test must be completed by the analyst and documented and approved by the DNA Technical Manager. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

4. If a laboratory generates new interpretation protocols from legacy validation data (e.g., developing a stochastic threshold when none previously existed), the laboratory shall document the basis for the new interpretation protocols. These new protocols shall be documented in the laboratory's standard operating procedures and memorialized in the Quality Assurance Standards Audit Document. The training and interpretation competency test must also be completed by the analyst and documented and approved by the DNA Technical Manager. Documentation shall contain sufficient

information for an auditor to confirm the analyst is qualified to reinterpret legacy amplification test kit data.

Technical reviewers of reinterpretations of the legacy data are held to the same training requirements as the analyst reinterpreting the data.

5. 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 and cleaning common areas. Protective gloves and a laboratory coat should be worn when cleaning the laboratory or instruments. The following maintenance and cleaning policies are in addition to the cleaning required by analysts prior to and after using any bench area or instrument for analysis.

WEEKLY DUTIES

- Monitor the operating temperatures of all freezers, refrigerators, and incubators. Log temperatures on the log sheets attached to instrumentation. 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 instrument the log is for and what time period it covers.

- Fill distilled water carboys, reagent alcohol bottles, 10-20% bleach solution bottles, and Liquinox detergent bottles.

- Wash glassware and dishes. Return washed dishes to their appropriate storage location.

5.1.1. Glassware Washing

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

MONTHLY DUTIES

- Restock and organize supplies and workstations.
- Wipe counters with 10-20% bleach solution.
- Wipe/dust all instrument exteriors with ethanol.
- Clean centrifuges with 10-20% bleach solution followed by distilled water.
- Wipe inside of hoods with 10-20% bleach solution and plastic/exterior surfaces with ethanol.
- Clean interior and exterior of drying boxes with 10-20% bleach solution.
- Wipe/dust all shelving units.
- Wash EZ1 and EZ2 cartridge racks and tip racks and QIAcube tip waste drawer inserts.
- Vacuum floors (except post-amplification room floor).
- Mop (or Swiffer) the floors using 10-20% bleach.
- Clean sinks.

Record the completion of cleaning tasks in the Monthly Cleaning Worksheets and submit them for scanning in the Maintenance Logs.

5.2. MAINTENANCE LOGS

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

Cleaning Logs	Location	Frequency
Lab Tech Area	Electronic	Monthly
Main Extraction/Purification & Exam Rooms	Electronic	Monthly
PCR Setup Room	Electronic	Monthly
Post-amplification Room	Electronic	Monthly

5.3. DISPOSAL OF HAZARDOUS WASTE

There are five waste streams within the Forensic Biology Unit, four of which should be handled as hazardous waste. These are as follows:

5.3.1. 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.

5.3.2. QIAGEN REAGENT WASTE (EZ1)
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.

5.3.3. 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.

5.3.4. 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.

5.3.5. NON-HAZARDOUS WASTE

Small volumes of blood that will dry within a reasonable amount of time can be disposed of in the regular trash. Acid phosphatase, LMG, and dry phenolphthalein test swabs can be disposed of in the regular trash. All plasticware (e.g., tips, tubes, transfer pipets, etc.) and gloves can be disposed of in the regular trash. Any non-hazardous waste liquid can be disposed of down the drain.

6. EQUIPMENT MAINTENANCE PROTOCOLS

6.1. OPERATION OF THE SMART2PURE WATER PURIFICATION SYSTEM

The Smart2Pure water purification system can prepare deionized water and PCR-grade water. A sterile filter must be attached to the outlet for the system to produce PCR-grade water; without the filter, it only produces deionized water. The water should have a conductivity of 0.055µS/cm (resistance of 18.2MΩ·cm). If the conductivity exceeds this value, it may indicate that the system has an improper setting or requires maintenance.

The Smart2Pure has a black rotary knob on the upper right-hand side of the front of the instrument. Rotating the knob counter-clockwise (toward the user) will start dispensing water from the outlet on the upper front of the instrument. Rotating the knob clockwise (away from the user) will stop dispensing water.

For troubleshooting, refer to the Smart2Pure User Manual

6.1.1. QUARTERLY MAINTENANCE

Quarterly maintenance includes a Concentrate flow adjustment:

1. Verify unit is in production mode (actively producing water), if not, dispense enough water to lower the float in the tank. The instrument will switch to production mode within a few minutes.
2. Place the end of the concentrate tubing into a graduated cylinder and collect the concentrate for 36 seconds
3. Measure the amount of concentrate collected. Divide this number by 10. This is the concentrate flow in liters per hour. *Concentrate flow should be 50 L/hr. Can adjust to as low as 30 L/hr if water production is too slow.
4. To adjust flow, remove cartridge cover and locate the pressure hold valve. Turn CCW to increase concentrate flow. Turn CW to decrease concentrate flow.
5. Remeasure concentrate flow for 36 seconds to check the rate.

6.1.2. SEMI-ANNUAL MAINTENANCE

Semi-annual maintenance should be performed in-house and includes the replacement of the pre-treatment filters. Pre-treatment unit filters should be purchased via Ariba and replaced approx. 6 months after Annual PM (or sooner—dependent on use rate).

1. Empty the storage tank and close the dispensing valve.
2. Disconnect from power supply.
3. Shut off feedwater.
4. Open the dispensing valve and close it when water no longer flows.
5. Remove pre-treatment filters by pressing on the quick connectors at the top and bottom of the cartridge and pulling the cartridges free.
6. Insert the new filter cartridges by aligning the quick connectors and pushing them into the connecting points until there is an audible click.
7. Turn on feedwater.
8. Reconnect to power supply.
9. Verify pressure gauges are showing desired readings.
10. The used filters/cartridges can be disposed in the regular trash.

6.1.3. ANNUAL MAINTENANCE

Annual maintenance should be performed by a service technician during annual preventative maintenance. The service technician should provide RO and UltraPure filters, pre-treatment unit filters and a dispenser final filter.

The annual maintenance should include the following steps:

- Disinfect Unit
- Replace RO Cartridge (10-451-034)
- Replace UltraPure Filter Cartridge (10-451-028)
- Replace Dispenser Final Filter (10-451-026)

1. Unplug from power supply.
2. Open the lid of the storage tank, pour the contents of a syringe of cleaning solution into the water-filled tank and close the lid.
3. Reconnect to power and let it run for 1 hour.
4. Discard two tank fillings of water.
5. When tank is empty, close the dispensing valve and disconnect from power supply.
6. Shut off feedwater.
7. Open the dispensing valve and close it when water no longer flows.
8. Remove cartridge cover.
9. Remove the UltraPure cartridge by pressing on the two quick connectors and pulling the cartridge free.
10. Remove the RO cartridge by pressing on the three quick connectors and pulling the cartridge free.
11. Insert the new RO and UltraPure cartridges by aligning the quick connectors and pushing them into the connecting points until there is an audible click.
12. Turn on the feedwater supply.
13. Reconnect to power supply.
14. Inspect the filter connections for leaks.
15. Replace cartridge cover.
16. Discard the first tank filling.
17. Replace the dispenser final filter.
18. The used filters/cartridges can be disposed in the regular trash.

6.1.4. REPLACING THE RO/ULTRAPURE CARTRIDGE

The RO/UltraPure Cartridge should be replaced during annual maintenance. These instructions are for situations when the cartridge needs replacement between annual maintenance visits.

1. Empty the storage tank and close the dispensing valve.
2. Disconnect from power supply.
3. Shut off feedwater.
4. Open the dispensing valve and close it when water no longer flows.
5. Remove cartridge cover.
6. Remove the UltraPure/RO cartridge by pressing on the quick connectors and pulling the cartridge free.

7. Insert the UltraPure/RO cartridge by aligning the quick connectors and pushing them into the connecting points until there is an audible click.
8. Turn on the feedwater supply.
9. Reconnect to power supply.
10. Inspect the filter connections for leaks.
11. Replace cartridge cover.
12. Discard the first tank filling.
13. The used filters/cartridges can be disposed in the regular trash.

6.1.5. REPLACING THE UV LAMP (10-451-025)

Wear gloves when handling the UV Lamp

1. Empty storage tank and unplug from power supply.
2. Shut off feedwater.
3. Remove cartridge cover.
4. Remove the UltraPure and RO cartridges.
5. Unscrew the two screws from the bracket of the UV assembly.
6. Pull the UV lamp out about 1 cm while still plugged in. Unplug the UV lamp.
7. Pull the UV lamp up and out while turning in a clockwise direction.
8. Check that the flat seal ring and o-ring of new UV lamp are properly seated.
9. Insert the new UV lamp while turning it in a counter-clockwise direction.
10. Plug in the UV lamp.
11. Screw UV assembly back into place.
12. Reattach the UltraPure and RO cartridges.
13. Reconnect to feedwater.
14. Reconnect to power supply.
15. Dispose of the used UV Lamp in the Universal Waste.

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.
   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 HNO3 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 Kimwipe.
7. Place electrode into first buffer.
8. Wait for **"Ready"** then record value in logbook.
9. Rinse the electrode in DI water and blot with a Kimwipe.
10. Place electrode into second buffer.
11. Wait for **"Ready"** then record value in logbook.
12. Rinse the electrode in DI water and blot with a Kimwipe.

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 Kimwipe.
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.

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

6.4.1. Laboratory balances will be calibrated and verified by an outside company once a year.

6.5. MAINTENANCE OF THE QIAGEN EZ1 and EZ2 instruments

Each month the piercing units will be cleaned and the O-rings greased on the QIAGEN EZ1 and EZ2 robots. Maintenance actions will be recorded on the QIAGEN EZ1 Maintenance Worksheet. A volume test will be 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.

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.
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 µL 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 500µL pipetted in the volume test, the allowable range is 474 to 525 µL. Recall that 500µL 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.
   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.
   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.

6.7. QIACUBE MAINTENANCE

6.7.1. MONTHLY MAINTENANCE

1. Clean the optical sensor, tip adapter, gripper unit, including the gripper, the stabilizing rod, and the spin column lid holder, by carefully wiping these modules with a soft lint-free cloth moistened with water. To gain access to the modules within the robotic arm:

    a. Press "Tools" in the main menu.

    b. Select "Maintenance" by pressing the up/down arrows to scroll through the list until it is highlighted, and then press "Select".

    c. Select "Cleaning position" by pressing the up/down arrows to scroll through the list until it is highlighted, and then press "Start".

    d. Follow the instructions displayed in the touchscreen. You will be instructed to remove the waste drawer and the labware tray.

    The robotic arm will move forward and downwards, enabling the modules to be accessed for cleaning through the opening for the waste drawer.

2. After cleaning the modules of the robotic arm, switch off the QIAcube at the power switch.

3. Thoroughly wipe the worktable with a soft lint-free cloth moistened with cleaning agent. Incubate as appropriate, rinse with distilled water, and wipe dry with paper towels.

4. Clean the shaker rack, labware tray, heating adapter, and reagent bottle rack with cleaning agent. Incubate as appropriate, rinse thoroughly with distilled water, and wipe dry with paper towels.

5. Clean the liner of the waste drawer with cleaning agent. Incubate as appropriate, rinse with distilled water, and wipe dry with paper towels.

6. Thoroughly wipe the inside and outside of the QIAcube using the cleaning agents.

Important: Do not use alcohol or alcohol-based disinfectants to decontaminate the QIAcube door.

Important: Take care that no liquid runs down the touchscreen. Liquid may be drawn through the dust protection sealing by capillary forces and cause malfunction of the display. To clean the touchscreen, moisten a soft lint-free cloth with 70% ethanol or a mild disinfectant and carefully wipe the display. Wipe dry with a paper towel.

6.7.2. SEMI-ANNUAL MAINTENANCE

The centrifuge lid must be open to allow access to the inside of the centrifuge. The lid should be opened only after the centrifuge has come to a complete stop. If the lid does not open automatically, perform the following steps.

1. Press “Tools” in the main menu.

2. Highlight “Maintenance” (scroll through the list by using the up/down arrows), and press “Select”.

3. Highlight “Open lid” (scroll through the list by pressing the up/down arrows), and press “Select”.

Cleaning the rotor and buckets

1. Switch off the QIAcube at the power switch.

2. Remove used disposable labware, sample tubes, and reagents from the worktable. Discard according to your local safety regulations.

3. Close the buffer bottles tightly, and store according to the instructions in the relevant kit handbook.

4. Remove all disposable rotor adapters, including tubes and spin columns, from the buckets.

5. Remove the buckets from the rotor. Undo the rotor nut on top of the rotor using the rotor key, and carefully lift the rotor off the rotor shaft.

6. Submerge the rotor, buckets, and rotor nut in cleaning agent. Incubate as appropriate.

7. Rinse thoroughly with distilled water. Use a brush (e.g., a toothbrush or tube brush) to clean any parts that are difficult to access, such as the bucket mount and the rotor head. Wipe surfaces dry with a soft lint-free cloth. If available, dry the buckets and rotor with pressurized air.

   Important: Make sure the paper towels and brush used are lint-free.

   Important: Make sure that all residual salt is removed.

   Important: Make sure to remove all traces of cleaning agent from the centrifuge buckets. Residual agent can cause the buckets to jam.

8. Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) on a soft, lint-free cloth, and wipe the bucket mount and rotor claw. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent.

9. Apply oil to the rotor claw and to the bucket mount.

Important: Before applying oil to the rotor buckets on the rotor, make sure that the rotor and all buckets are completely dry.

Cleaning the centrifuge

1. Moisten a soft lint-free cloth with cleaning agent and clean the inside of the centrifuge and the centrifuge gasket. Incubate as appropriate.

2. Clean the inside of the centrifuge and the gasket with distilled water and wipe dry with lint-free paper towels. If available, use a vacuum cleaner.

Important: Make sure the gaskets remain in the proper positions.

3. Clean the centrifuge lid with a soft lint-free cloth moistened with cleaning agent. Incubate as appropriate, clean with water, and wipe dry with paper towels.

4. Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.

Installing the centrifuge rotor and buckets

1. Mount the rotor. The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position 1. Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft. Install the rotor nut on top of the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated.

2. Replace the rotor buckets. When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely.

Important: All centrifuge buckets must be mounted before starting a run.

Tightness Test

The tightness test is performed to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.

1. Load an empty 2 ml safe- lock microcentrifuge tube in position 1 of the shaker.

2. Fill a reagent bottle with 96% ethanol and place in position 1 of the reagent bottle rack.

3. Load a tip rack of the filter tips you want to test (1000 µl or 1000 µl wide-bore) onto the QIAcube.

4. Make sure that the QIAcube is switched on.

5. In the main menu, press “Tools”.

6. Select “Maintenance” by pressing the up/down buttons to scroll through the list until it is highlighted, and then press “Select”.

7. Select “Tightness test” by pressing the up/down buttons to scroll through the list until it is highlighted, and then press “Select”.

8. Select the appropriate type of filter-tips (“1000 µl tips” or “1000 µl wide-bore tips”) by pressing the up/down buttons to scroll through the list until it is highlighted, and then press “Select”.

9. Press “Start” to start the tightness test with the selected type of filter-tips.

10. Follow the instructions displayed in the touchscreen, and press “Start” to start the tightness test. After the load check, the robotic arm will pick up a tip, aspirate ethanol, and move to the tube. The tip will remain in place above the tube for 2 minutes. The tip will be detached.

11. After the protocol is completed, open the QIAcube door and check if the tube contains liquid. If the tube is still empty and dry, the

tightness of the pipetting system is adequate. If you find liquid in the tube, contact QIAGEN Technical Services.

6.7.3. ANNUAL MAINTENANCE

A QIAGEN Service Engineer will perform maintenance on an annual basis.

6.8. QUANTSTUDIO 5 CALIBRATION / VERIFICATION PROCEDURES

The information for the procedures detailed in this section was taken from the QuantStudio™ 5 Real-Time PCR Instrument (for Human Identification) User Guide.

6.8.1. QUANTSTUDIO 5 – REGIONS OF INTEREST CALIBRATION

MATERIALS REQUIRED:

1. Region of Interest (ROI) and Background Calibration Plates (Part No. 4432364)

2. 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.

Note:
The ROI Calibration invalidates all other calibrations in section 6.8. Following ROI calibration, procedures 6.8.2 through 6.8.4 must be performed.

Preparing the Plate:

Retrieve the ROI Calibration plate from the ROI and Background Calibration Plates kit in the amplified DNA room freezer. Allow the plate to thaw for at least 30 minutes. Protect the plate from light until use.

Leave the optical film on the plate. (Do not discard the packaging as the plate can be used up to three times within 6 months after opening). Vortex and centrifuge the thawed plate to assure that the liquid is in the bottom of the plate wells.

Performing the ROI Calibration

1. From the instrument home screen, navigate to **Settings** > **Maintenance and Service** > **Calibrations** > **ROI and Uniformity**
2. Follow the instructions on the screen to start the ROI calibration.
3. Load the plate on the instrument
4. Touch **Start**
5. When the run is complete and the screen displays **Calibration Complete**, touch **View Results** to check the calibration status.
6. If the calibration status is "Passed", touch **Next** to proceed to the Background Calibration (see procedure 6.8.2), Dye Calibrations (procedures 6.8.3 and 6.8.4), and RNase P Verification (procedure 6.8.5).
7. If the calibration status is "Failed", consult the "Troubleshoot calibration failure" section of the User Guide.
8. Remove the plate from the QuantStudio 5 instrument and return the plate to the packaging. If the ROI plate has not been used three times, you may return the plate to the freezer.

6.8.2. QUANTSTUDIO 5 – BACKGROUND CALIBRATION

MATERIALS REQUIRED:

1. Region of Interest (ROI) and Background Calibration Plates (Part No. 4432364). If Background Calibration Plate is unavailable, a plate prepared with 50µL of PCR grade water in each well and sealed with an optical adhesive cover may be used.

2. Centrifuge and vortex

Purpose:
A background calibration measures the level of background fluorescence in the instrument. The software can then use the information to remove the background fluorescence from the run data.

Note:
The Background Calibration requires a current ROI Calibration. If the ROI calibration is required, perform procedure 6.8.1 prior to the Background Calibration.

Preparing the Background Calibration Plate:
Retrieve the prepared Background Calibration plate from the ROI and Background Calibration Plates kit from the freezer in the amplified DNA room. If Background Calibration Plate is unavailable, a plate prepared with 50µL of PCR grade water in each well and sealed with an optical adhesive cover may be used. Allow the plate to thaw, if necessary (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.

Performing the Background Calibration

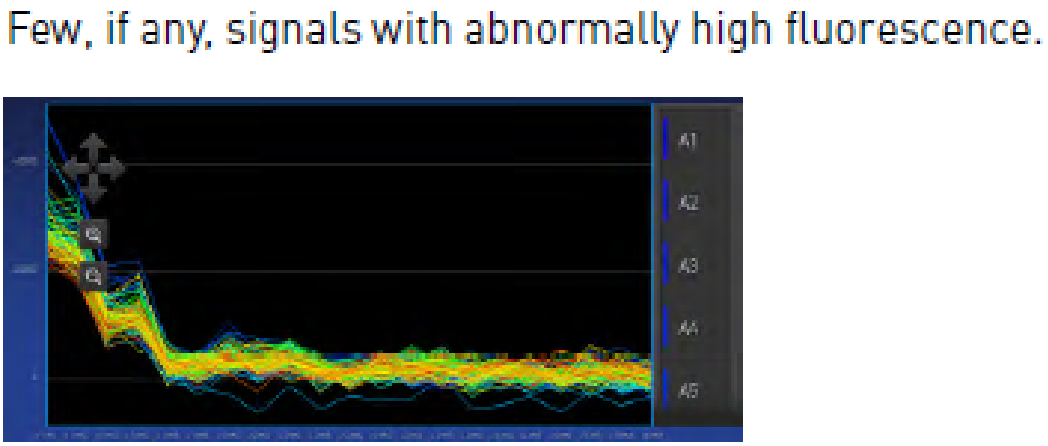
1. From the instrument home screen, navigate to **Settings** > **Maintenance and Service** > **Calibrations** > **Custom** > **Background**

2. Follow the instructions on the screen to start the Background Calibration.

3. Load the plate on the instrument

4. Touch **Start**

5. When the run is complete and the screen displays **Calibration**



Complete, touch **View Results** to check the calibration status.

6. If fluorescence is noted in certain wells, remove the plate from the instrument, rotate it 180°, and return the plate to the instrument. Rerun the Background Calibration.

    a. If fluorescence is noted in the same positions as previously obtained, the sample block is contaminated. See the decontamination of the sample block procedure in the User Manual

    b. If fluorescence is in the reverse positions as previously obtained, the plate is contaminated. Discard the plate and rerun background calibration with a new plate.

7. If the calibration status is "Passed", touch **Next**

8. If the calibration status is "Failed", consult the "Troubleshoot calibration failure" section of the User Guide.

9. Remove the plate from the QuantStudio 5 instrument and return the plate to the packaging. If the Background Calibration plate has not been used three times, you may return the plate to the freezer.

6.8.3. QUANTSTUDIO 5 – SYSTEM DYE CALIBRATION

MATERIALS REQUIRED:

1. QuantStudio 3/5 10-Dye Spectral Calibration Kit (Part No. A26343)
2. Centrifuge and vortex

Purpose:
The system dye calibration characterizes and distinguishes the individual contribution of each dye in the total fluorescence signal collected by the instrument.

Note:
The Dye Calibration requires a current ROI Calibration and a current Background Calibration. If the ROI Calibration and/or Background Calibration is required, perform procedures 6.10.1 and/or 6.10.2 prior to the Dye Calibration.

Preparing the Plate:
The 10-Dye Spectral Calibration Kit includes three plates of spectral calibration standards. Retrieve the appropriate plate from the 10-Dye Spectral Calibration Kit in the amplified DNA room freezer. Allow the plate to thaw for at least 30 minutes. Protect the plate from light until use. Leave the optical film on the plate. (Do not discard the packaging as the plate can be used up to three times within 6 months after opening). Vortex and centrifuge the thawed plate to assure that the liquid is in the bottom of the plate wells.

Performing the System Dye Calibration

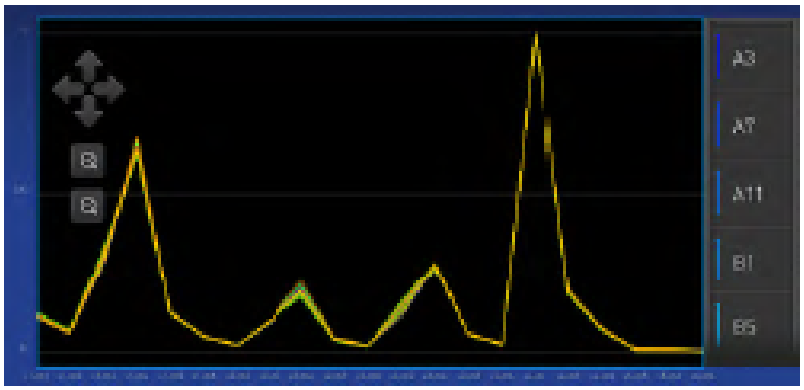
1. From the instrument home screen, navigate to **Settings** > **Maintenance and Service** > **Calibrations** > **Dye**
2. Follow the instructions on the screen to start the System Dye Calibration.
3. Select the appropriate dye plate that you are running and touch **Next**

4. Load the plate on the instrument

5. Touch **Start**

6. When the run is complete and the screen displays **Calibration Complete**, touch **View Results** to check the calibration status.

Signals from each well following a uniform trend, and each dye peaks at the correct filter.



7. If the calibration results are acceptable, touch **Accept Results**. Touch **Next** to perform System Dye Calibration of the remaining plate(s) in the kit.

   a. Remove the plate from the QuantStudio 5 instrument and return the plate to the packaging. If the Dye Calibration plate has not been used three times, you may return the plate to the freezer.

   b. Repeat steps 1-7 for the remaining calibration plate(s) in the kit

8. When all three plates have successfully run and the results have been accepted, touch **Next** to proceed to HID Dye Calibration (see procedure 6.10.4).

9. If the calibration results do not meet expectations "Troubleshoot calibration failure" section of the User Guide.

6.8.4. QUANTSTUDIO 5 – HID DYE CALIBRATION

MATERIALS REQUIRED:

1. ABY Spectral Calibration Plate (Part No. 4461591)
2. JUN Spectral Calibration Plate (Part No. 4461593)
3. Centrifuge and vortex

Purpose:
The ABY and JUN dyes used for HID applications like Quantifiler Trio are slightly different than the dyes used on the system calibration plates. These calibrations characterize and distinguish the individual contribution of these dyes in the total fluorescence signal collected by the instrument.

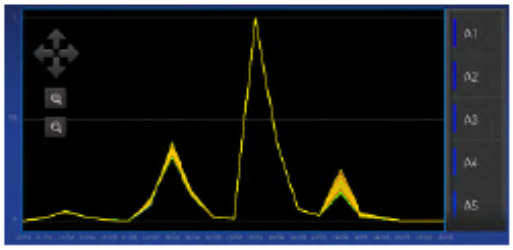
Note:
The Dye Calibration requires a current ROI Calibration and a current Background Calibration. If the ROI Calibration and/or Background Calibration is required, perform procedures 6.10.1 and/or 6.10.2 prior to the Dye Calibration.

Preparing the Plate:
Retrieve the appropriate plate from the amplified DNA room freezer. Allow the plate to thaw for at least 30 minutes. Protect the plate from light until use. Leave the optical film on the plate. (Do not discard the packaging as the plate can be used up to three times within 6 months after opening). Vortex and centrifuge the thawed plate to assure that the liquid is in the bottom of the plate wells.
.

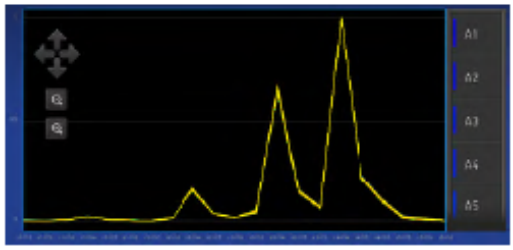
Performing the HID Dye Calibration

1. From the instrument home screen, navigate to **Settings** > **Maintenance and Service** > **Calibrations** > **Custom** > **Custom Dye**
2. Follow the instructions on the screen to start the Dye Calibration.
3. Select the appropriate dye plate that you are running and select **Next**
4. Load the plate on the instrument
5. Touch **Start**
6. When the run is complete and the screen displays **Calibration**

Dye	Peak filter	Filter wavelength (nm)	
		Excitation	Emission
ABY-HID	x3-m3	550 ± 10	587 ± 10
JUN-HID	x4-m4	580 ± 10	623 ± 14



ABY-HID dye calibration plot



JUN-HID dye calibration plot

Complete, touch **View Results** to check the calibration status.

7. If the calibration results are acceptable, touch **Accept Results**. Touch **Next** to perform System Dye Calibration of the remaining plate(s) in the kit.
    a. Remove the plate from the QuantStudio 5 instrument and return the plate to the packaging. If the HID Dye Calibration

plate has not been used three times, return the plate to the freezer.

b. Repeat steps 1-7 for the remaining calibration plate in the kit.

8. When both plates have successfully run and the results have been accepted, touch **Next**.

9. If the calibration results do not meet expectations "Troubleshoot calibration failure" section of the User Guide.

6.8.5. QuantStudio 5 – TaqMan RNase P Verification

MATERIALS REQUIRED:

1. TaqMan RNase P Instrument Verification Plate (Part No. 4432382)

2. Centrifuge and vortex

Purpose

The TaqMan RNase P Instrument Verification Plate run verifies the performance of the QuantStudio 5 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 software will assess the instrument performance.

Note:
The RNase P verification requires a current ROI Calibration, Background Calibration, and dye calibrations. If the ROI Calibration and/or Background Calibration and/or Dye Calibrations is required, perform the applicable procedures 6.10.1 through 6.10.4 prior to the RNase P Verification.

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.

Performing the HID Dye Calibration

1. From the instrument home screen, navigate to **Settings** > **Maintenance and Service** > **RNase P Verification**
2. Load the plate on the instrument
3. Touch **Start**
4. When the run is complete and the screen displays **Verification Complete**, touch **View Results** to check the status of the run.
5. If the calibration status is "Passed", the instrument is ready for use. In the **RNase P Verification Status** screen, touch **Accept Results**.
6. If the calibration status is "Failed", consult the "Troubleshoot calibration failure" section of the User Guide.
7. Remove the plate from the QuantStudio 5 instrument and discard it.

6.9. PROCEDURE FOR THE CALIBRATION CHECKS OF THE THERMOCYCLERS

The thermal cyclers 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 thermal cycler approximately every six months. The verifications are conducted by an outside vendor.

6.10. 3500 MAINTENANCE

OVERVIEW OF 3500 MAINTENANCE SCHEDULE:

Weekly/Biweekly (recommended in the following order):
Run the Wash Pump and Channels Wizard (weekly)
Clean anode buffer cup pin-valve assembly (weekly)
Place new Anode Buffer Container on instrument **(Hard Stop)** (bi-weekly)
Replace Cathode Buffer Container **(Hard Stop)** (bi-weekly)
Restart computer and instrument (weekly)

Monthly:
Flush the Pump Trap
Replace septa on Cathode Buffer Container
Clean the autosampler and drip tray
Defragment Hard drives

As needed:
Replace Polymer
Spatial Calibration
Spectral Calibration
Replace Capillary Array (also requires spatial and spectral)
Pump cleaning

Powering on the 3500 and launching the Data Collection software

1. Power on the computer. At the Log On prompt, log in to the computer.

2. Before turning on the 3500 instrument, ensure that the oven door is closed and locked, that the instrument doors are closed, and the computer is powered on.

3. Power on the instrument by pressing the on/off button on the front of the instrument. Ensure that the green status light is on and not flashing before proceeding.

4. Click on the **3500 Series Data Collection Software 2** icon on the desktop. Log in to the data collection software with the password taped to the monitor.

5. Use the Dashboard to review maintenance notifications. When you complete a task, click the **green check mark** to indicate that it was completed, or the **red X** to mark it as dismissed.

6. Click on Maintenance on the top panel. Use the left panel to navigate to the schedule calendar or Maintenance Wizards to complete the needed maintenance.

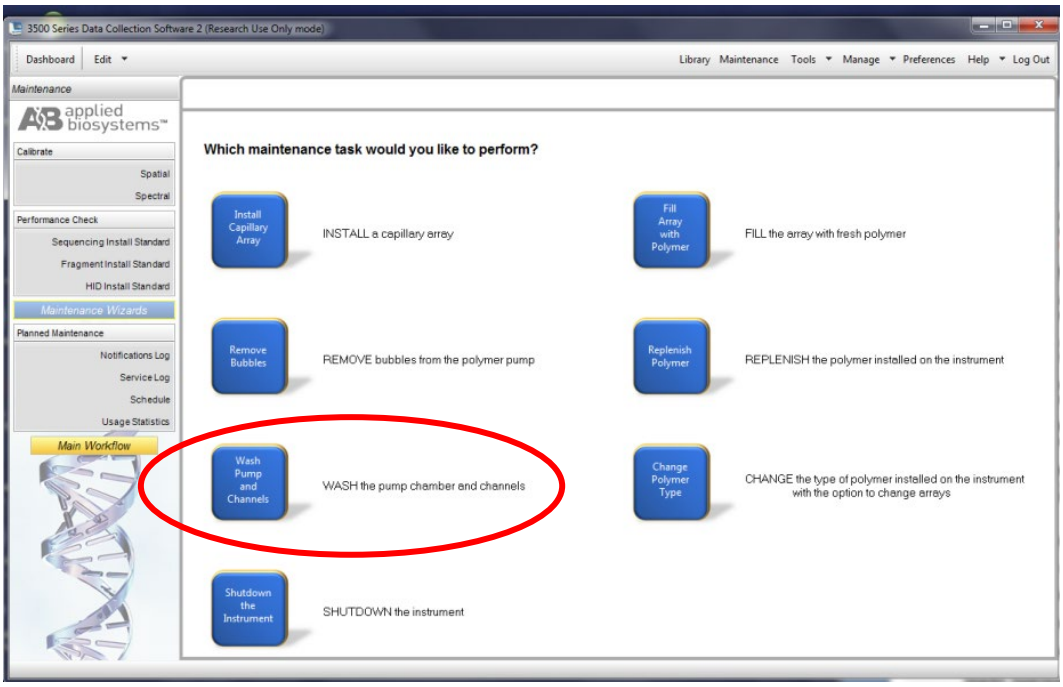
Weekly Maintenance

At least 30 minutes prior to beginning weekly maintenance, bring Conditioning Reagent, POP-4 Polymer*, Anode buffer container, and Cathode Buffer Container to equilibrate to room temperature.

*If polymer remains before the expiration date, and there are still injections left in the pouch, polymer can be reused for a total of approximately four weeks on the instrument before it needs to be replaced.

Run the Wash Pump and Channels Wizard

This wizard takes about 40 minutes from start to finish. Click '**Maintenance**' on the top panel, and '**Maintenance Wizards**' on the left to find the '**Wash Pump and Channels**' wizard.



Follow the wizard prompts to empty the anode buffer (NOTE: The *EMPTY* anode buffer container is placed back on the instrument for the water wash), remove polymer, insert conditioning reagent, and insert polymer to flush the pump chamber and channels.

Clean anode buffer container valve pin assembly

Following pump chamber and channels wash, and before installing the new anode buffer container, use a clean lint-free cloth and deionized water to wipe clean the anode buffer container valve pin assembly on the polymer delivery pump.

Place new Anode Buffer Container on instrument

1. Remove Anode Buffer Container from outer plastic wrap. Ideally, this is done after the wash pump and channels wizard.

2. Tilt it slightly to make sure that most of the buffer is in the larger side of the container. There should be less than 1 mL of buffer remaining in the small side of the container. Verify that buffer is to the fill line.

3. Gently peel off the plastic seal on the top without spilling any into the small side of the container. If it does spill into the overfill side, pipette it back into the reservoir so that the liquid reaches the fill line.

4. Place the Anode Buffer Container (label side first) under the valve pin assembly, and slowly slide the lip of the container into the locks.

Replace Cathode Buffer Container (replacing septa only once per month)

1. Remove Cathode Buffer Container from outer plastic wrap and peel off plastic seal from the top, ensuring that it is peeled off of every circular opening.

2. Wipe off any buffer on top of the container with a lint-free cloth. Ensure that the top of it is dry.

3. Press **tray** on the front of the instrument to bring the autosampler forward. Remove the old cathode buffer by pinching in the middle.

4. Place the appropriate cathode buffer septa on both sides of the cathode buffer container. These only need to be replaced once per month. Septa can be washed with water and returned to the new cathode buffer.

5. Install the new buffer by clicking it into the tabs on the autosampler.

6. Close the instrument door to re-initialize.

Restart computer and instrument

1. Close data collection software.

2. Shut down computer.

3. Once computer is shut down, press the **power** button on the instrument.

4. Restart computer, but don't log in.

5. Once the computer is on, turn the 3500 back on by pressing the **power** again. Let the instrument initialize and wait for the solid green light to appear before logging on to the computer.

6. Click on the **3500 Series Data Collection Software 2** icon on the desktop. Log in to the data collection software (it may take a minute for the connections to be reestablished before the software can open).

Monthly Maintenance

Flush the Pump Trap

The water trap must be flushed once per month (or more often as needed) to prolong the life of the pump by cleaning any diluted polymer and clearing air bubbles.

1. Fill the supplied 20 mL, all-plastic Luer lock syringe with PCR-grade water. Do not use a syringe smaller than 20 mL. Doing so may generate excessive pressure within the trap. Expel any bubbles from the syringe.
2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

3. Open the Luer fitting by grasping the body of the fitting and turning it to loosen. Attached syringe and turn counterclockwise approximately one half turn.

IMPORTANT! Do not use excessive force when you push the syringe plunger as this may damage the trap seals. Take approximately 30 seconds to flush 5 mL of PCR-grade water through the trap.

4. Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

5. Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

6. Dispose the excess water inside the overflow container.

Replace septa on Cathode Buffer Container

Once per month, change both of the septa on the Cathode Buffer Container during the weekly maintenance.

Clean the autosampler and drip tray

Wipe them down with a lint-free cloth and either deionized water or absolute ethanol

Defragment hard drives

Click the **Windows Start icon** to find **Disk Defragmenter** and open it. Click on each disk and click '**Analyze**' to determine the degree of fragmentation. Click '**Defragment**' for every disk that is fragmented. This may take several minutes to hours depending on the degree of fragmentation.

As Needed Maintenance

Replace Polymer

If there isn't sufficient polymer to complete your run or if the polymer has exceeded 2 weeks use on the instrument, it is necessary to place a new polymer pouch on the instrument. Get a new polymer pouch and let it equilibrate to room temperature before replacing the old one. Use the **Replenish Polymer wizard** to complete this. Follow the wizard prompts in order to flush the pump of the old polymer and refill the capillaries with the new polymer. This wizard takes 10-20 minutes to complete.

Spatial Calibration

The 3500 Series Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal is detected by the CCD camera. A spatial calibration is required to ensure correct alignment of the capillaries and optimal detection of the signal in each capillary.

A new spatial calibration is recommended when:

- A capillary array is installed, replaced or temporarily removed from the detection block
- The instrument is moved
- The detection cell door is opened

Perform Spatial Calibration and Evaluate Results

1. Click '**Maintenance**' on the Dashboard. Click '**Spatial**' under the Calibrate heading on the left.

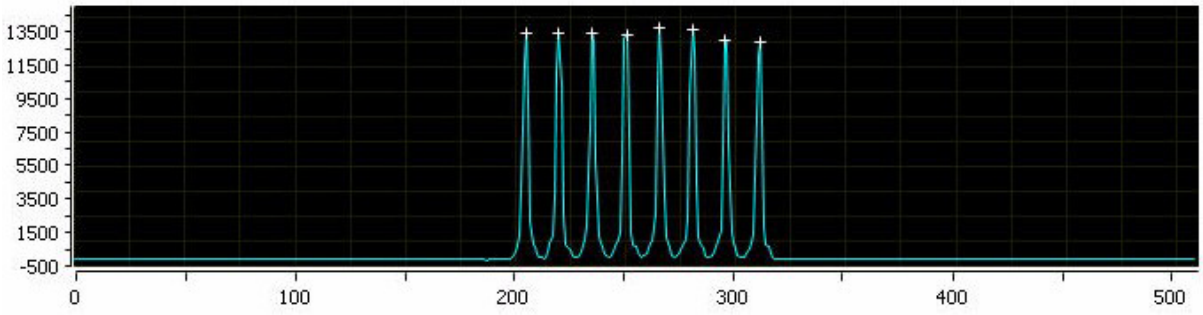
2. Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.

3. Click '**Start Calibration**'. The display updates as the run progresses.

4. When the run is complete, evaluate the spatial calibration profile to ensure that you see the following:
   - One sharp peak for each capillary. Small shoulders may be acceptable.
   - One marker (+) at the apex of every peak. No off-apex markers.
   - An even peak profile (all peaks about the same height).
   - The Max Spacing – Min Spacing should be no more than 2 pixels.

5. If the results meet the criteria above, click '**Accept Results**'.

Note: If any peaks are lower than usual for the instrument or the heights drastically slope up or down, repeat the calibration with a fill. See below for an example of an acceptable Spatial Profile.



6. Click '**View Spatial Calibration Report**'.

Spectral Calibration

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to effectively correct for spectral overlap and minimize the presence of artifacts, such as spectral pull-up, in the data.

A new spectral calibration is recommended/required when:

- The capillary array is changed (required) or temporarily removed from the detection block.
- The instrument is moved.
- The laser or CCD camera has been realigned/replaced by a service engineer.
- An increase in (pull-up and/or pull-down peaks) is observed.
- A new dye set is used on the instrument.
- The capillary array length or polymer type is changed.
- The detection cell door is opened (not required by the software, but recommended).

Perform Spectral Calibration and Evaluate Results

1. Pre-heat the oven by clicking '**Start Pre-heat**'.

Note: Preheating for approximately 30 minutes helps mitigate subtle first-run migration rate effects. The pre-heat function automatically turns off after 2 hours.

2. Click '**Maintenance**' on the Dashboard. Click '**Spectral**' under the Calibrate heading on the left panel.

3. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed.

4. Vortex the appropriate Matrix Standard. Prepare the Master Mix:

For GlobalFiler® and Yfiler™ Plus analysis, use **Dye Set J6.**
   a. Standard: 10 µL
   b. HIDI Formamide: 290 µL

Note: Volumes other than above may be used to obtain spectral peaks between 3000-10,000 RFU. The total volume should not exceed 300uL.

Note: After a capillary array is changed, a new spectral calibration must be run for each dye set.

5. Load 10 µL of matrix standard/HIDI mixture into wells A1-H1 of a 96-well reaction plate. Note: multiple matrix samples (e.g., J6 or G5) can be run in subsequent columns on this plate if needed)

6. Cover the plate with a 3500 septa mat and centrifuge to ensure that the samples are at the bottom of the wells and place the reaction plate in the Veriti® thermal cycler.

7. Denature the samples by running the **spectraldenature** protocol on the Veriti thermalcycler (95˚C, 5 min).

Note: The cover plate of the Veriti® thermal cycler may be closed onto the septa mat-covered reaction plate, but do not clamp the cover down.

Although the lid is not heated during the denature protocol, it may stick to the septa mat and pull it up when the cover is opened if it is clamped down.

8. After the denaturation protocol is finished, chill the reaction plate immediately in the 96-well cooling block (-20˚C) for at least 3 minutes.

9. Place the plate into a 3500 Series 96-well plate base (it will only fit in one orientation) and cover with a 3500 Genetic Analyzer 96-well plate retainer. Check to ensure that the retainer clip is properly seated.

10. Press the **tray** button on the front of the 3500 Genetic Analyzer. When the autosampler tray presents itself, open the door of the instrument and place the tray onto the either position of the autosampler (the tray will only fit in one orientation). Tilt the plate toward the middle, first before lowering into the side clip. Close the instrument door.

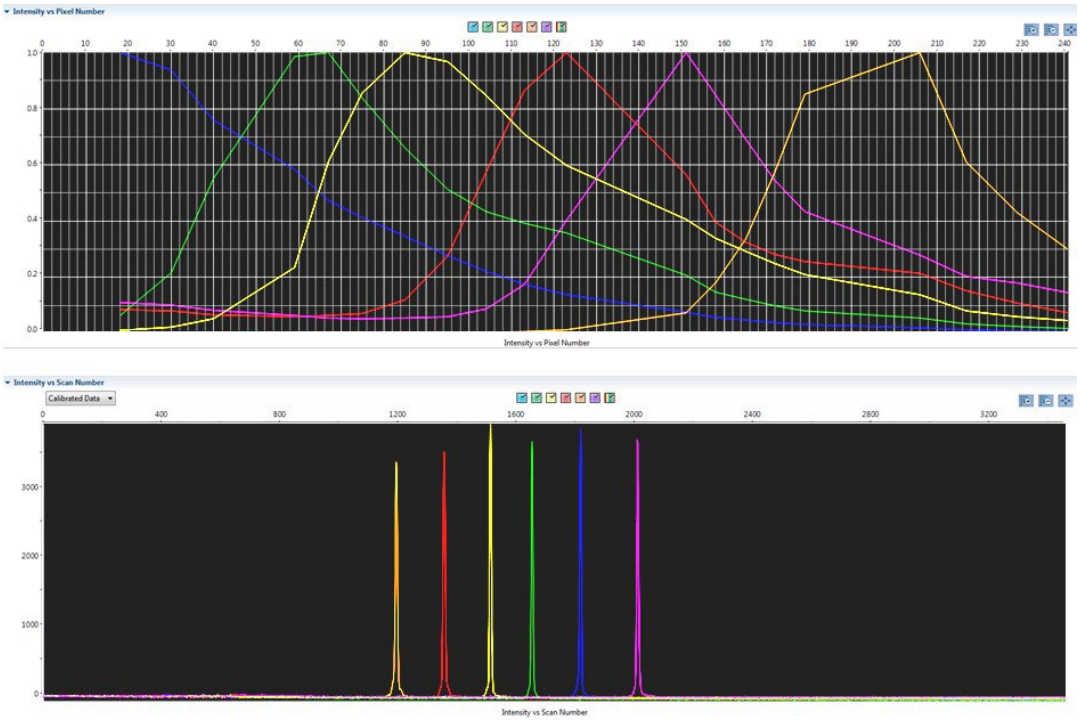
11. In the 3500 Series Data Collection Software 2, Click '**Maintenance**' on the dashboard. Click '**Spectral**' under the Calibrate heading on the left.

12. Select the number of wells (96), plate position, chemistry standard (Matrix Standard) and dye set (J6 or G5). Ensure that 'Allow Borrowing' and 'Perform Run 2\Run3 if Run 1 Fails' are deselected.

13. Click '**Start Run**'. Run takes ~30 minutes to complete. Passing capillaries are shown in green and failing capillaries are shown in red. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed.

14. Evaluate each capillary for the following:
    - Order of the peaks in the spectral profile from left to right: J6: blue, green, yellow, red, purple, orange (an example is below)

- Peak heights are sufficiently robust (>2,000 RFU) and do not contain extraneous peaks, gross overlaps, dips, or irregular morphology. If peak heights exceed 20,000 RFU, set up another plate with a reduced amount of matrix standard. If peak heights are below 2,000 RFU, set up another plate with an increased



  amount of matrix standard. See below for an example of an acceptable J6 spectral profile. G5 is similar, but without the purple.

15. If the results meet the criteria above, click '**Accept Results**'. If the results do not meet the criteria, set up another plate, adjusting amount of matrix standard, if necessary, and run again.

Installing or replacing the capillary array

It is recommended that the capillary array be replaced after 160 injections per capillary, or removed from the instrument for storage if the instrument is to be shut down for an extended period of time; however, capillary life may be extended based on the quality of the data obtained from the array.

Select the '**Install Array Wizard**' for guidance. This wizard takes 15-45 minutes to complete.

Note: A spatial calibration AND spectral calibration are required after a capillary array change. The instrument will not run without a new spectral calibration associated with that capillary RFID tag.

Removing bubbles from the pump block

Periodically, check the pump chamber, channels and tubing for air bubbles. Press the button on the right hand side of the front of the instrument to turn on the lamp inside the instrument. This will help locate any air bubbles.

Select the '**Bubble Removal Wizard**' for guidance. This wizard takes 5-15 minutes to complete.

Pump Cleaning

In nearly all circumstances, the Water Wash Wizard is very effective in cleaning the pump assembly. Occasionally, a situation in which the Water Wash Wizard is not sufficiently effective may arise. If polymer has dried in the channels of the lower block, or if some contamination in the pump assembly is suspected, use the Pump Cleaning Kit to thoroughly clean the pump assembly.

If this procedure is needed, refer to the Pump Cleaning Kit Protocol for 3500 Series Analyzers.

REFERENCES:
Applied Biosystems 3500/3500xL Genetic Analyzer User Guide
Applied Biosystems 3500/3500xL Genetic Analyzer User Bulletin
Product insert sheet from Dye Set J6 (Pub. Number 4426042 Rev. A)
Pump Cleaning Kit Protocol for 3500 Series Analyzers

7. COLLECTION KITS AND SUPPLY ORDERING

7.1. PREPARATION OF EVIDENCE COLLECTION KITS

The Forensic Biology Unit provides officers, investigators, and sexual assault nurses with three types of kits used in the collection of biological evidence.

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):

- 1 pair of large nitrile gloves
- 2 sterile, paper-wrapped swabs
- 1 small "SDPD Reference Mouth Swabs" envelope
- 1 smaller manila envelope
- 1 evidence seal
- 1 copy of 'Instructions for the Collection of Reference Mouth Swabs'
- 1 copy of the SDPD consent forms and expungement forms

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):

- 1 pair of large nitrile gloves
- 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 copy of 'Instructions for the Collection of a Biological Stain'

7.1.3. 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
- 1 pair of large nitrile gloves

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

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\Forensic Biology\Laboratory Technician\Ordering, Quotes, POs\Forensic Biology 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 laboratory-designated lot number (e.g., GF#-YY).

7.2.4.1. QUANTIFILER TRIO KITS

The Quantifiler Trio Kit contains a cardboard sleeve with two attached stickers around two smaller boxes. One box contains the DNA standard and the other box contains the PCR reagents. The DNA standard should be removed from its box and placed into the box containing the PCR reagents. Record lot numbers of the PCR reaction mix, DNA standard, primer, and whole kit in the Kit Receipt Log. One of the two stickers with the kit lot number on them should be affixed to the PCR reagents box. The kit (in the PCR Reagents box) will be stored in the reagent freezer. Place "Not QC'd, Do Not Use" stickers on the kit. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

7.2.4.2. STR TYPING KITS

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 laboratory-designated lot number and stored in the reagent refrigerator. The ladder will be labeled with the laboratory-designated lot number and stored in the reagent freezer 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 EZ2)

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.

7.2.4.3.1. Several strips from the currently QC'd lot and several strips from the new lot should be set aside for comparison during QC process.