SAN DIEGO POLICE DEPARTMENT

FORENSIC SCIENCE SECTION

FORENSIC BIOLOGY UNIT POLICY MANUAL

All Procedures Reviewed and Approved by: _____ **Date: 03-01-2010**

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UNIT DESCRIPTION

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

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

UNIT FUNCTIONS

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

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

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

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

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

PERSONNEL AND JOB DESCRIPTIONS

SUPERVISING CRIMINALIST

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

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

A. Establishes casework priorities based on negotiations and discussions with investigators and district attorneys.

B. Assigns casework to analysts.

C. Reviews reports and examines case notes.

D. Reviews and evaluates the performance of subordinates.

E. Works with the DNA Technical Manager to review and establish Unit specific policies and analytical procedures when appropriate.

F. Interviews and hires new employees.

G. Trains the new employees on department policies and procedures.

H. Works with the DNA Technical Manager to coordinate analyst training.

I. Keeps track of workload statistics.

J. Prepares budget requests.

K. Holds Unit meetings to disseminate department, laboratory, or unit specific information.

L. Prepares reports as required by the Crime Laboratory Manager.

DNA TECHNICAL MANAGER

The DNA Technical Manager will oversee the technical operation of the DNA Laboratory as outlined by the DNA Advisory Board. The DNA Technical Manager will evaluate new technologies, resolve technical problems, assess and design training programs, and ensure the unit adheres to all quality assurance guidelines. The DNA Technical Manager is authorized to shut down the DNA laboratory should a serious technical problem develop.

Duties and responsibilities:

A. Responsible for the technical operation of the laboratory as outlined by the DNA Advisory Board.

B. Reviewing, revising and approving any DNA-related technical policy or procedure prior to final approval by the QA Manager.

C. Approving and overseeing training of new employees (when applicable) in DNA analytical procedures, including completion of training documentation.

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

E. Working with the QA Manager on any QA reports involving a DNA technical issue, and reviewing and signing off on those QA reports.

F. Acting as a technical reference for the QA Manager.

G. Performing some technical reviews of DNA case files.

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

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

J. Working with Unit Supervisor and QA Manager to ensure compliance of the DNA analysts with QA and Unit policies and procedures.

K. Reviewing the DNA proficiency tests completed by the Forensic Biology Unit.

L. Conducting casework analysis.

M. Prepares reports as required by the Crime Laboratory Manager.

CASEWORK CODIS ADMINISTRATOR

The Casework CODIS Administrator is responsible for the security and integrity of the local CODIS network, the CODIS software, and the data within the local database. The Casework CODIS Administrator ensures the data within the local CODIS database is in compliance with NDIS guidelines. If the Casework CODIS Administrator determines at any point that the CODIS network, software, or entered data has been compromised, the DNA Technical Manager will be immediately notified. The DNA Technical Manager and Casework CODIS Administrator will determine the proper course of action, which ultimately may include shutting down the CODIS network.

Duties and responsibilities:

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

B. Ensures that DNA profiles are searched against the local CODIS database.

C. Uploads data to the California CODIS database.

D. Downloads and searches DNA profiles from the CJIS WAN website.

E. Performs upgrades to the CODIS software with the assistance of the CODIS Help Desk.

F. Ensures that a backup is maintained for the information in the local database.

G. Assists in the training of analysts in the operation of CODIS software.

H. Disseminates CODIS information to the rest of the Forensic Biology Unit.

CRIMINALIST (I AND II)

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

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

Duties and responsibilities:

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

B. Locates and identifies biological material.

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

D. Writes reports summarizing the findings and provide expert testimony in court.

E. Remains technically current and knowledgeable in appropriate analytical methods.

F. Participates in the evaluation and validation of new technologies or methodologies.

G. Participates in any special projects as assigned by the Supervising Criminalist or Laboratory Manager.

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

I. Uses the technical manual, unit policies manual and general quality assurance manual to deal with any procedural question or analytical method.

LABORATORY TECHNICIAN

The Laboratory technician functions as a support position for the Criminalists in the Forensic Biology Unit. Duties include making all reagents, quality control testing critical reagents and kits before application to casework, ordering supplies, calibrating critical 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. Certain functions described above may be performed by volunteers or interns but only under the direct supervision of the laboratory technician.

Duties and responsibilities:

A. Prepares reagents as needed for biological fluid identification and DNA testing.

B. Performs quality control checks on critical reagents and DNA typing kits.

C. Checks the calibration of instruments such as pipettes, thermal cyclers, and balances.

D. Orders chemicals and supplies for Forensic Biology

E. Maintains an accurate inventory of all chemicals located in the Unit.

F. Maintains water purification system.

G. Ensures that laboratory instruments, communal work areas, and floors are cleaned and decontaminated.

H. Ensures that the operating temperatures of the Unit ovens, incubators, refrigerators, and freezers are monitored.

I. Ensures that victim, suspect, and physiological, and reference mouth swab kits are prepared for departmental use or for the collection of evidence at area hospitals.

J. Conducts casework screening as assigned by the Supervising Criminalist.

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

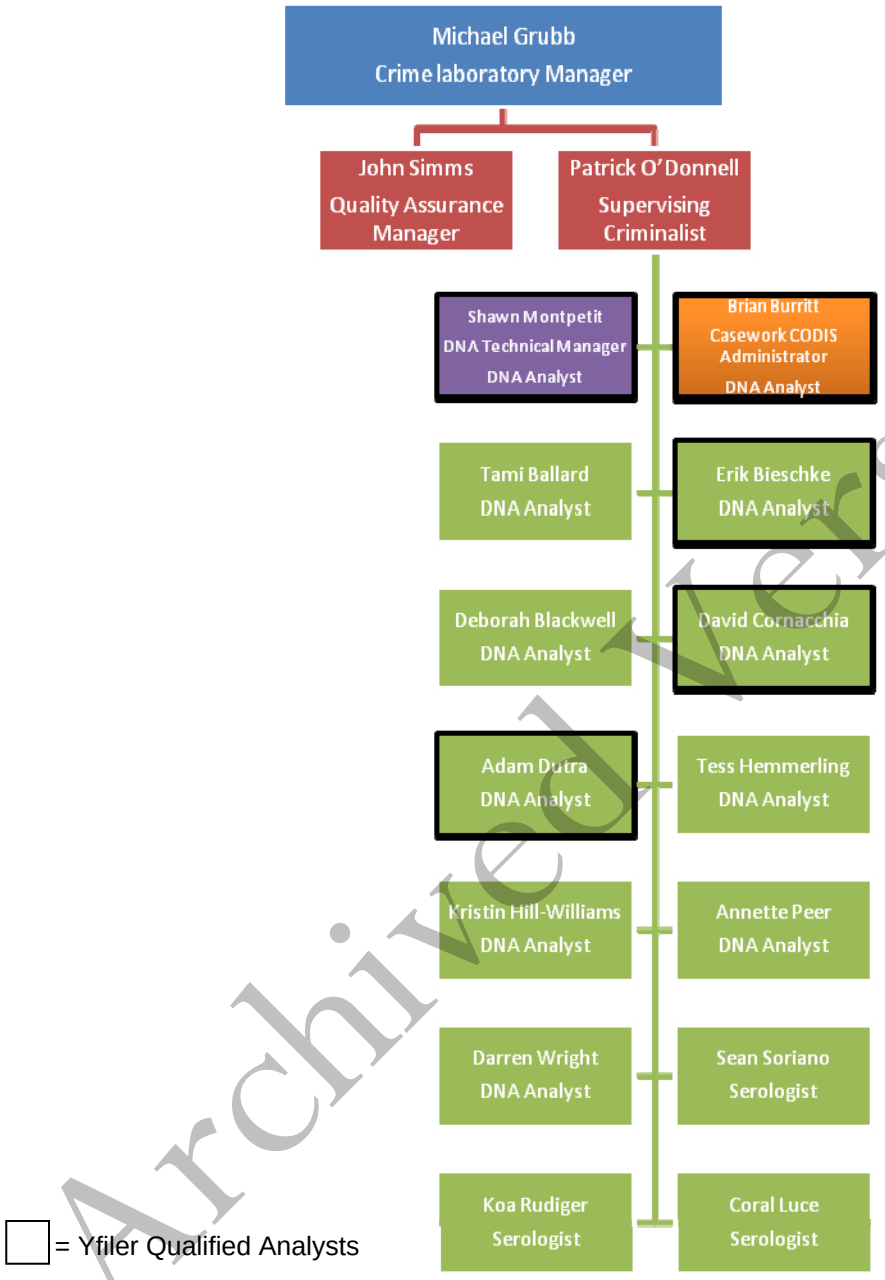
INTERN/VOLUNTEERS

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

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

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

ORGANIZATIONAL CHART FOR FORENSIC BIOLOGY



TECHNICAL LEAD CONTINGENCY PLAN

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

QUALITY ASSURANCE REVIEWS AND REPORTS

In general, Quality Assurance Reviews will be conducted when policy or method non-compliances are identified in administrative practices, evidence handling, casework, or in proficiency test situations. Additionally, a Quality Assurance Review will be conducted on any incident of Staff DNA Contamination or in any unexpected DNA results in controls that compromise the interpretation or results.

If a non-compliance is found to exist, then a Quality Assurance Report will be issued. Additionally, a Quality Assurance Reports will be issued on any incident of Staff DNA Contamination or in any unexpected DNA results in controls that compromise the interpretation of the results.

Please see policy 7.5 “Noncompliance review and corrective action protocol” in the General Quality Assurance Manual for further information

UNEXPECTED RESULTS IN CONTROLS

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

Any unexpected results will be documented by the analyst conducting the analysis. The analyst will complete an "unexpected result summary" [*template located on the FB network in the Misc Data folder*] for inclusion in the "Unexpected Result Log". This log will be maintained by the DNA Technical Manager. The DNA Technical Manager and the Supervising Criminalist of the Forensic Biology Unit will be notified of any unexpected results, review, and sign off on the summary. If the problem is deemed serious then the Quality Assurance manager will be notified.

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

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

A Quality Assurance Report will be issued when any unexpected DNA results in a control compromise the interpretation of the results.

FORENSIC BIOLOGY UNIT INCIDENT LOG

The FB Unit Incident Log will monitor unusual events that occur during casework that :

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

The unusual incidents will be documented by the analyst conducting the analysis. The analyst will complete an "Incident Summary" [*template located on the FB network in the Misc Data folder*] for inclusion in the "FB Incident Log". This log will be maintained by the DNA Technical Manager. The DNA Technical Manager and the Supervising Criminalist of the Forensic Biology Unit will be notified of any unusual incident, review, and sign off on the summary.

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

Supporting documentation, if available, will be provided with the summary.

QUALITY CONTROL OF CRITICAL REAGENTS

The following list contains the reagents deemed critical to the DNA testing process and consists of both, solutions prepared in-house, as well as commercial kits purchased from outside vendors.

All reagents used in the organic extraction of DNA (excluding dithiothreitol (DTT))
Qiagen DNA Investigator Kits
Quantifiler qPCR Kits
Identifiler STR Typing Kits
Bulk Taq Gold DNA Polymerase
Yfiler DNA Typing Kits
Minifiler DNA Typing Kits

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

Samples available for Quality Control Testing
Only samples with known DNA profile results will be used to assess critical reagents. The samples currently available for quality control testing are the SAM and PTO dried blood samples and the JM and PTO extracted DNA samples that are stored frozen. The current batch of extracted JM and PTO samples (Extract Date: 10/17/2000) have been made NIST traceable.

Strategy for testing

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

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

The critical reagents/kits will be tested as follows:

Extraction Reagents: Two different QC 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. This worksheet and the supporting data will be maintained in the DNA Extraction – Quality Control of Critical Reagents Binder.

Quantifiler kits: New kit lots will be tested using previously extracted known DNA samples. The samples will be carried through amplification with Identifiler. Target volume of the QC samples will be determined based on the quantification value obtained from the new Quantifiler Kit. Successful typing of the known samples will serve to demonstrate the reliability of the new kit lot(s).

Qiagen DNA Investigator kits: Two different QC samples and a reagent blank will be carried through DNA extraction and typing. The kit, reagent strip lot numbers, and the results of the DNA testing will be documented on the Quality Control Worksheet for DNA Investigator kits. This worksheet and the supporting data will be maintained in the Qiagen Robot Reagent QC binder.

Identifiler™ DNA typing kits: Two known samples will be typed along with the 9947A positive control and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for Identifiler. This worksheet and the supporting data will be maintained in the Identifiler QC binder. (See also the NIST Testing policy below)

Bulk Taq Gold DNA Polymerase: Two known samples will be amplified along with the 9947A positive control and an amplification blank using previously QC'd Identifiler reagents and the bulk Taq DNA polymerase. The lot number(s) of the bulk Taq DNA polymerase and the results of the DNA testing will be documented on the Quality Control Worksheet for Bulk Taq DNA Polymerase. This worksheet and the supporting data will be maintained in the Bulk Taq QC binder.

Yfiler DNA typing kits: New kit lots will be tested with two known samples [the PTO sample (Extraction Date: 10/17/2000) has been made NIST traceable], the 007 DNA positive amplification control, and an amplification blank. Alternatively the new kit lots may be tested with the NIST 2395 SRM 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. This worksheet and the supporting data will be maintained in the Y-STR QC binder.

Minifiler DNA typing kits: Two known samples will be typed along with the 007 DNA positive control and an amplification blank. The lot numbers of all kit components and the results of the DNA testing will be documented on the Quality Control Worksheet for Minifiler. This worksheet and the supporting data will be maintained in the Minifiler QC binder.

NIST TESTING OF DNA PROCEDURES

Once a year, the DNA typing process will be checked against NIST or NIST-traceable standards. The previously extracted known DNA samples (JM and PTO) have been tested alongside NIST SRM samples thus making them NIST traceable. In the future when the current batch of known samples run out, a subsequent batch of samples will be extracted and typed alongside the NIST SRM to make the new batch NIST traceable as well.

PROFICIENCY TESTING

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

Each DNA analyst in the Forensic Biology Unit must complete two proficiency tests in each calendar year with an interval between the tests of no less than four months and no more than eight months. Both of these proficiency tests will be external and will involve body fluid identification and DNA typing. Each screening analyst will be proficiency tested once per calendar year. For DNA proficiency tests, the due date of the proficiency test (as listed by the test provider) will be the date by which these tests are tracked.

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

DNA analysts are required to perform at least one organic and one automated DNA extraction per year for the proficiency tests. Both methods may be used on a single proficiency test. Identifiler and Yfiler (for those qualified) must be run on each semiannual proficiency test. Minifiler must be run on proficiency test samples at least once each year.

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

LITERATURE REVIEW

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

QUALITY ASSURANCE LOGS FOR THE FORENSIC BIOLOGY UNIT

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

Log Name	Location
Kit Receipt Log	Main FB
Reagent Preparation Logs	Main FB
Supply Order Log	Main FB
Screen and Freeze Reagent Preparation Log	Main FB
Amplification Room Solution Log	Amplification Room
DNA Extraction -Quality Control of Critical Reagents	CODIS
Qiagen Robot Reagent QC	CODIS
Identifiler QC	CODIS
Y-STR QC	CODIS
Unexpected Results Log	CODIS
pH Meter Calibration	Main FB
Balance Calibration Tests	Main FB/Amp Room
Pipette Calibration Log	Main FB/Amp Room
9700 Thermal Cycler Calibration & Uniformity Log	Amplification Room
Ovens & Fridges Temperature Log	Main FB/Amp Room
Qiagen EZ1 Volume Test	Main FB

REPORT FORMAT

The Biology Unit will use the standardized report format located in the Quality Systems Manual.

In general, items described in the report will be limited to those examined or tested.

CONTENTS OF FINAL CASE PACKETS

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

Communication records (telephonic or written) will be documented and retained as either numbered pages of the case notes or as administrative documents.

Numbering of administrative documents as part of the analytical record will be at the analyst's discretion. All additional documents in the case packet (e.g. administrative documents) will include the case number and analyst's initials on the first bound page or on all unbound pages.

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

RELEASE OF PRELIMINARY RESULTS

The analyst working on a case may verbally release preliminary results. The analyst must make it clear that the results are preliminary and final results may be reported differently based on further analysis (refer to the INFORMATION DISCLOSURE policy 10.1, in the Quality Systems Manual).

DISTRIBUTION OF FINAL REPORTS

After final signature by the analyst, technical reviewer, and administrative reviewer, the analyst will ensure the report for is distributed as follows:

- The original copy of the report will be filed with the case packet in the laboratory's main case files. The Clerical Staff will be responsible for filing the finalized case packets.
- The requesting detective will receive an electronic copy (or photocopy) of the final report.

- An additional electronic copy of the report will be maintained on the Forensic Biology Unit computer network so that an easily accessed archive of all reports issued exists.
- A copy (electronic or other) will be sent to Records for scanning into the Central Records Management System (CRMS). Homicide cases and cases with Lab Sequence numbers are exceptions to this.

INTERPRETATION

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 in 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. 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 sensitive 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.

Fluid identification test results:

Identification of blood

Catalytic test	HemaTrace	Conclusions
Negative	Not Done	No blood was found
Positive	Not Done	Presumptive test indicated the possible presence of blood.*
Positive	Negative	Presumptive test indicated the possible presence of blood; however the presence of human blood could not be confirmed.
Positive	Positive	Blood was found.**
Negative	Positive	Blood was found.**

*Analysts may come to the reasonable conclusion that a stain is apparently blood (to be stated in report as such) assuming the stain appears visually consistent with the analyst's expectation for blood, a positive catalytic test result, or the stain's existence is obvious given the context of the crime. An example would be a bloodstained t-shirt belonging to a stabbing victim. A positive HemaTrace result is mandated for an analyst to conclude blood (or human blood) is present if or when the stain is diffuse, the catalytic test result for the stain is suspect because it is difficult to visualize, the stain is on a substrate, such as rust, known to possibly cause a weak positive reaction with the catalytic test, or triggers a weak catalytic test. Analyst's are encouraged where there is any doubt, or where the probative value of stain is critical, to employ the HemaTrace test to conclusively demonstrate the presence of blood.

**May also be blood of ferret or higher primate in origin. This statement must appear in report or testimony as qualifier if conclusion is presented as to human blood being present.

Identification of semen

Catalytic Test	Sperm	p30	Conclusion
Negative	Negative	Not Done	No semen was found.
Negative	Negative	Negative	No semen was found.
Positive	Negative	Negative	No Semen was found.
Negative	Positive	Negative	Sperm cells were found.
Positive	Positive	Not Done	Semen was found.
Positive	Negative	Positive	Seminal fluid / semen was found.
Negative	Positive	Positive	Semen was found.
Positive	Positive	Positive	Semen was found.
Positive	Positive	Negative	Semen was found.
Positive/Negative	Inconclusive	Inconclusive	Inconclusive
Positive/Negative	Inconclusive	Inconclusive/Negative	Inconclusive

DNA controls:

Reagent blanks

The reagent blank is a check for possible contamination of the sample preparation reagents by extraneous DNA. If DNA types are detected in the reagent blank this is an indication of a possible contamination event or that extraneous DNA was introduced into the sample from some unintended source. Based on an evaluation of the DNA types in the reagent blank the results of associated samples may be deemed inconclusive. Samples extracted in a batch where the reagent blank shows DNA types greater than the interpretation threshold may need to be re-extracted.
Not all contamination events will necessitate reanalysis and will be judged on a case-by-case basis.

In some cases, re-extraction of samples affected by a reagent blank containing detectable DNA may not be possible. Results for these samples may be reported if any possible concerns related to the reagent blank are adequately explained.

Amplification blank ("no DNA" control)

The amplification blank is a check for possible contamination samples during the amplification process. The appearance of DNA types in this control indicates that problems similar to those described for the reagent blank may have occurred during the amplification.

The interpretation of detectable DNA in the amplification blank should take into account the results obtained from any samples and reagent blanks amplified in the same batch. A batch where both the amplification blank and the reagent blank give the same typing result, or where only the amplification blank gives a result, may be caused by a problem limited to the amplification process. In this case, re-amplification of the samples may resolve the problem. A batch that shows a typing result for the reagent blank, but not for the amplification blank is more likely to be caused by an extraction-related problem. If the reagent blank and amplification blank give different typing results, both the extraction and amplification processes are potentially suspect and should be repeated.

Positive amplification control

The positive amplification control is a check for effectiveness of the amplification and the Short Tandem Repeat (STR) analytical processes. This control should always show interpretable results consistent with the known genotype published in the DNA kit literature. If the positive amplification control does not produce the expected results, any samples associated with this control will be deemed inconclusive.

Samples in the batch associated with the failed positive amplification control that have DNA remaining will be re-amplified.

For samples associated with failed positive amplification control that were consumed for the original amplification the most appropriate remediation would be to re-examine (re-extract) the original evidence. If no evidence remains then the samples will be reported as inconclusive.

The only time the results from samples associated with failed positive amplification control that were consumed for the original amplification will be reported is when they form the basis of an exclusion. In this instance, troubleshooting of the problem must lead to the determination that the amplification for that sample is reliable and full disclosure of the unexpected result (failed positive amplification control) as well as the exclusion will be documented in the final report.

For samples associated with a reagent blank that was consumed, if the troubleshooting of the failed positive amplification control demonstrates that the results for the reagent blank can be relied upon, then the results from that control can be used to demonstrate the extraction reagents were clean for any re-amplified sample. If troubleshooting cannot determine that the results from the reagent blank are reliable then the most

appropriate remediation for any sample associated with that reagent blank would be to re-examine (re-extract) the original evidence. If no evidence remains then the samples will be reported as inconclusive.

CASEWORK REVIEW

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

Technical review

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

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

The analytical record serves as the basis for all conclusions set forth in the final report and will be evaluated in the technical review process. The analytical record will be examined by the technical reviewer to ensure that it is complete and that current Forensic Biology Unit procedures (including interpretation guidelines) and policies for the examination and testing of biological evidence were followed.

Unresolved differences between the case analyst and the reviewing analyst can be resolved by seeking the opinions of other qualified analysts in the Forensic Biology Unit, or by consulting the DNA Technical Manager.

TECHNICAL REVIEW EXPECTATIONS

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

1. The Analyst and Technical Reviewer will meet in person and have a **verbal** conversation in an effort to sort out any differences of opinion. Each will have the opportunity to professionally explain their point of view. This conversation should in many cases resolve the issue.
2. Assuming the Analyst and Technical Reviewer cannot come to an agreement the input of a mutually agreeable Arbitrating Analyst will be sought. This analyst must be qualified in the technical area being discussed.
3. If the issue raised in Technical Review has policy implications or is seen as a particularly difficult issue by any of the parties involved, the Technical Manager will serve the role of Arbitrating Analyst.

4. Should the issue raised in technical review have policy implications, there may need to be a discussion of the situation in a Unit meeting. The Technical Manager will work with the Supervising Criminalist to set the agenda for this discussion. The policy ramifications may also be so broad as to mandate involvement of the Quality Assurance Manager to clarify general laboratory policy.
5. Assuming an agreement between the Analyst, the Technical Reviewer, and Arbitrating Analyst (Technical Manager in many instances) cannot be achieved, the Quality Assurance Manager will be asked to assist the three parties in coming to a resolution for the technical issue.
6. Should the technical review issue still remain unresolved the Supervising Criminalist for Forensic Biology will be informed of the impasse.
7. Analysts involved in the discussion may elect to have further discussions with the Laboratory Director concerning the technical issue.

The review approval will be documented by having the reviewing analyst initial and date both the report and the first page of the analytical record.

Administrative review

Each case packet prepared by an analyst is subject to administrative review by the Supervising Criminalist of the Forensic Biology Unit. In the event the Supervising Criminalist is unavailable the administrative review may be performed by another Analyst within the section.

The purpose of the administrative review is to ensure that established protocols are being applied to case analysis, the report format is acceptable, the appropriate wording has been used, the chain of custody is documented, and the analytical record is complete.

CASE SUBMISSIONS AND HANDLING

CASE SUBMISSIONS

The Forensic Biology Unit aspires to a turnaround time of twenty working days for most casework requests submitted. This is a performance-based budgeting goal.

WORK REQUESTS

Work requests are logged into the laboratory's computerized case tracking system by the Clerical Unit and then forwarded to the unit supervisor for prioritization, assignment, and tracking. If a request for analysis is received directly from a detective or inter-office mail, the request (or a copy of it) will be forwarded to the Clerical Unit to be entered into the tracking system.

CASE TRACKING

The unit supervisor is responsible for entering case information into the computerized case tracking system. The information tracked by the unit supervisor is the case number, the analyst a work request was assigned to, the date it was assigned, the date it was completed, and the number of items the analyst performed work on.

CASEWORK STATISTICS

Casework statistics are recorded for each case that is analyzed. A case statistics worksheet is submitted to the unit supervisor by the analyst when work on a case has been completed. These numbers are tallied in monthly statistics for the Unit. Statistics are tallied for work performed on the number of items of evidence in a given case.

CONSUMPTION OF EVIDENCE

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

In Homicide, Sex crimes, and Child abuse cases where no suspect is associated with a case, the analyst will consult with the unit supervisor prior to consuming the sample. These consultations/communications will be documented in the case notes. The analyst is encouraged to contact the case detective to inform them of the decision to consume the sample(s).

In cases other than homicide, sex crimes, and child abuse, if no suspect is listed, an analyst may consume the sample(s) in an attempt to gain investigative information.

Discussion:
Homicide or Sex Crime case with charged suspect: If a need to consume the evidence exists you must contact the attorney and it is recommended to inform the detective of your intent to consume the evidence. The attorney will consult with the defense and the laboratory may be asked to retain the evidence for an independent laboratory to do joint testing.

Homicide, Sex Crimes, or Child Abuse cases with no suspect: The analyst is encouraged to inform the detective that in our judgment we need to consume the evidence. The detective may ask to put a hold on the testing if they possess information that we do not. The Supervisor will be consulted in these cases before the detective is contacted.

Lesser Felony case with a suspect: If in the judgment of the analyst it provides the best chance of obtaining a DNA profile, the detective will be informed of our intent to consume the evidence. This keeps the detective up to speed with our analysis and provides the detective an opportunity to provide additional details concerning the case.

Lesser Felony case without a suspect: Analyst is free to consume the evidence if in their judgment it provides the best chance of obtaining a DNA profile.

In all instances we are the best judge of what needs to be consumed for DNA testing and are not seeking permission but rather are keeping the interested parties informed of what is taking place as a courtesy.

UNIT POLICIES FOR EVIDENCE HANDLING

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

General:

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

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

- Prior to analysis, the work area must be cleaned with a 10-20% bleach solution. The work area will be covered with clean paper or bench mats. The contents of items must be inventoried and examined on a fresh piece of paper. This paper can be used to wrap the item after examination, and should be marked with the item's identifying information.

- All items examined must be marked with the case number, date, and analyst's initials. If circumstances prevent or preclude marking the item directly only their proximal containers will be marked. Any analysts performing verifications need only mark their initials on an item.

- When evidence to be analyzed is retrieved from the Property Room or Laboratory Vault the condition of packaging is noted (i.e., damaged, unsealed, etc). When opening an item, care must be taken to avoid compromising any written information on the package. If possible the original seal will not be broken. If the contents of the packaging cannot be accessed without breaking the seal, then a notation will be made in the case notes.

- The analyst will wear appropriate safety gear and disposable gloves.

- The sequence of examination must be determined. Communication between units is important in accomplishing this. A unit able to individualize evidence has priority over a unit that may only be able to determine class characteristics. Destructive examinations will be carried out as a last resort.

- When possible, avoid consuming an entire evidence sample for analysis. Where possible, a portion of the original evidence should be retained for future testing. See the FB policy on evidence consumption as well as the Laboratory Quality Systems Manual for the Laboratory policy on evidence consumption.

- The danger of cross-contamination must be recognized and steps taken to prevent it. Evidence collected from the suspect must not be analyzed or extracted at the same time as evidence collected from the victim. The DNA extraction of evidence and reference standards must be separated by time and/or place.

- If slides or other exemplars are generated as a result of analysis, a notation as to final disposition will be made in the notes. It must also be noted if freezer packets are made or evidence is transferred.

- Digital images will be taken of most evidence and color laser prints of these images will appear in the case notes. In addition, an archive of all digital images taken by Forensic Biology analysts is maintained on the FB network.

- After analysis, items will be returned to the original packaging if possible. The package must be sealed with evidence tape and the analyst must initial and date the seal. The case number, property tag number, and the item number must be on the outer packaging.

- For any procedure performed in the Forensic Biology Unit only one evidence sample tube should be open at a time during any sampling and reagent/DNA addition. The automated processes employed in the Forensic Biology Unit are exceptions to this policy.

- Within the Forensic Biology Unit there are separate refrigerators and freezers dedicated to the storage of either reagents or evidence. Evidence and reagents should be stored separately in their designated locations while in the Forensic Biology Unit.

DNA extraction:

- When sampling for DNA analysis, a clean cutting surface should be used for each piece of evidence. Clean scissors or scalpel blades thoroughly with bleach, soap and water, alcohol, or by flaming after cutting each evidence sample. Alternatively, use a fresh scalpel or razor blade with each evidence sample.

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

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

- Reagents will be stored as small aliquots to minimize the effects of any contamination events. The lot(s) of reagents used for each set of analyses will be recorded so that if contamination occurs, it can be traced more readily.

- Sterile disposable aerosol resistant pipette tips and sterile tubes will be used for any processes involved in DNA analysis. Pipette tips will be changed between adding and removing liquid from evidence samples.

- It is advisable to briefly centrifuge all tubes before opening to remove liquid from the caps.

- The number of samples processed at one time should be limited to a manageable number. This precaution will reduce the risk of a sample mix-up and the potential for sample to sample contamination. The manageable number of samples will vary depending on the experience and comfort level of an analyst.

- The DNA extraction/purification of questioned samples will be performed separately from the DNA extraction/purification of reference samples from the same case. This precaution will help to prevent potential cross-contamination between reference and evidence samples. In addition, it is advisable that DNA extraction/purification of samples, where high amounts of DNA are expected, (references or large bloodstains) be performed separately from samples where low amounts of DNA are expected (single hairs or very small stains) to minimize the potential for sample to sample contamination.

- Only unamplified DNA samples will be stored in the refrigerators and freezers in the extraction area. No reagents used in the post amplification processes will be stored in any refrigerator and freezer in the DNA Extraction Area.

- At least one reagent blank will be extracted with each set of evidence sample extractions/purifications to check for possible contamination of the DNA extraction reagents or DNA Extraction Area. In many instances it may be appropriate to run multiple reagent blanks to accommodate the combining of evidence sample extracts or additional testing of evidence samples. The reagent blank(s) should be treated in the same manner as the most manipulated evidence sample associated with it. The reagent blank will be carried through amplification and analysis with its associated samples. If multiple reagent blanks are extracted, analysts are required to carry, at a minimum, the reagent blank with the highest quantitation value through to completion of analysis. If the reagent blank(s) associated with an extraction set are depleted then no testing can be performed on additional different amplification test kits.

PCR Setup:

- Use dedicated pipettes for adding kit reagents and DNA to the PCR tubes.

- Always add DNA to the PCR tube last. This reduces the chances for inadvertent

transfer of DNA between samples and kit reagents.

- Only one evidence sample tube should be open at a time during reagent and DNA addition.
- The negative amplification control (no DNA) allows for the detection of contamination during PCR setup.
- Avoid touching the inside surface of the tube caps.
- Change pipette tips after the addition of each DNA sample.
- Store the amplification kits in the designated reagent refrigerator. Do not store them near extracted DNA.

Amplified DNA:

- Only disposable lab coats are to be used in the Amplified DNA Area.
- The Amplified DNA Area is dedicated to amplified DNA and should not be used for pre-amplification purposes.
- Caution should be used when handling PCR products to minimize dispersing of the product in the Amplification Area.
- Always remove gloves and lab coats before exiting the Amplification Area.
- Change gloves whenever contamination with PCR product is suspected.
- Use disposable bench paper to cover work areas.
- All surfaces in the Amplification Area should be cleaned with 10-20% bleach solution before and after setting up samples for analysis. The floor of the Amplification Area will be mopped with a dilute bleach solution every two weeks.
- All equipment found in the Amplification Area is dedicated to that area and will never be removed unless proper decontamination procedures have been employed.

Store amplified DNA in the designated amplified DNA freezer. Under no circumstances should the amplified DNA be stored within the DNA Extraction Area.

DNA SAMPLE STORAGE

Extracted DNA

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

Extracted DNA will be stored long-term frozen in the Property Room. If a new Property Tag is created for the extracted DNA then a copy of that Property Tag must be included in the case notes. If extracted DNA is to be stored under an existing Property Tag then an Additional Item Form reflecting the creation of this freezer packet must be included in the case notes.

Amplified DNA

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

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

POLICIES REGARDING FREEZER PACKETS

Freezer packets are created to store derivative evidence separated or prepared from other evidence that is customarily stored unfrozen. If a freezer packet is created for derivative evidence, the freezer packet should be stored frozen.

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

-Property Tag number
-Victim's name
-Case number
-Item number(s) of the evidence contained within

An ADDITIONAL ITEMS FORM should be completed for each freezer packet created. The freezer packet(s), along with the ADDITIONAL ITEMS FORM, will be submitted to the Property Room for storage. A copy of the ADDITIONAL ITEMS FORM will be maintained in the case notes.

UNIQUE IDENTIFIERS

General:

All items analyzed from a given Property Tag are required to have a unique identifier when they are examined. The unique identifier for evidence items will be the case number (or lab sequence number), property tag number, and item number. Certain items of evidence may have a previously designated item number such as the case with homicide evidence where item numbers are detailed in the evidence list for a given case. Blood or urine samples will have had a B (blood) or U (urine) number assigned to them that will be used as the unique identifier for the sample.

Samples contained in microcentrifuge tubes:

During the screening and DNA analysis of evidence it is common to have samples contained in ~~small~~ microcentrifuge tubes. These include question stains, reference samples, cell pellets being preserved for possible DNA analysis, and extracted DNA. In addition, amplified product produced during the polymerase chain reaction process is also maintained for a period of six months. Each sample analyzed will be labeled with a unique identifier while it is in the FB unit. All microcentrifuge tubes containing question or reference samples, cell pellets, extracted DNA, or amplified DNA will be labeled with the case number and an appropriate sample identifier chosen by the analyst.

Additional descriptors may be employed to identify particular samples such as “NS” to identify non-sperm fractions of extracts. Where possible the sample label will be carried over and used as the CODIS profile identifier. Examples follow:

97-023647 4ANS: case number 97-023647, sample 4A, non-sperm fraction

98-032125 CT: case number 98-032125, reference sample from CT (e.g. Charles Thomas)

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

Any stains, cuttings, or swabs taken of items that will be impounded into the Property Room as additional evidence will fall under the sub-item numbering section of the Item Numbering policy (QA 5.2) and shall be numbered as dictated by that policy when they are impounded into the Property Room.

Method: **INSTRUCTIONS FOR COLLECTING BIOLOGICAL STAINS USING SWABS**

Date: 01/09/2007 Approved by: SAM

The following procedure can be used to collect most biological stains including blood, semen and saliva.

MATERIALS:

1. Plastic dropper bottle filled with sterile water.

2. Cotton tipped swabs with a wood applicator.

3. Manila envelopes.

PROCEDURE:

Small Stains

1. Being careful not to touch the swab with the dropper bottle, place a single drop of water on the end of a cotton swab. It may not be necessary to wet the swab if the stain is still wet.

2. Collect the stain using the very tip of the swab. Do not roll the swab. The small amount of material should be concentrated at the tip of the swab.

3. Place the swab in a manila envelope. The wooden stick may have to be partially broken off. The manila envelope should have all the necessary information concerning the collection of the swab.

Large Stains

1. Being careful not to touch the swab with the dropper bottle place two or three drops of water on the end of a cotton swab. It may not be necessary to wet the swab with water if the stain is still wet.

2. Collect the stain by rolling the swab in the stain so that a large amount of stain is collected on the swab. If possible, collect at least two swabs of the stain.

3. Place the swab(s) in an envelope. The wooden stick(s) may have to be partially broken off. The manila envelope should have all the necessary information concerning the collection of the swab.

REMARKS:

Control swabs of a stain free area may be collected whenever possible.

When wetting the swabs with distilled water, enough water will be applied to the swabs to make them moist but not so much that the swabs are soaked.

Stains will only be collected with swabs only if the stained substrate itself cannot be collected (e.g. stain on pavement or structure).

Method: **INSTRUCTIONS FOR PROCESSING FIREMARMS FOR DNA**

Date: 01/09/2007 Approved by: SAM

OPTION A: SWABBING FOR DNA FIRST

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

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

OPTION B: FINGERPRINT PROCESSING FIRST

In instances where the majority of the firearm has smooth surfaces, the Crime Scene Unit will process the firearm prior to swabbing for potential DNA evidence. The firearm will be visually examined and then processed with cyanoacrylate ester. A second visual examination will then be conducted to determine if there are any visible prints or ridge detail. If no ridge detail is present, the firearm will then be swabbed for DNA.

If the firearm will be processed using black powder or other chemical processing prior to swabbing it for potential DNA evidence then a previously unused brush and previously unused black powder will be used to avoid any possible transfer of DNA to the firearm. After the application of black powder, and any prints are lifted, the firearm can then be swabbed for DNA.

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

CODIS

Introduction

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 database primarily consists of casework evidence profiles, termed Forensic Unknowns, and convicted offender profiles. Additional sample categories include Suspects, Unidentified Human Remains, Missing Persons, and Relatives of Missing Persons. The purpose of CODIS is to develop investigative leads by matching DNA profiles of two or more samples from these various categories.

Entry of DNA Profiles into CODIS

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

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

- DNA profiles selected for entry into CODIS are submitted on forms referred to as CODIS sheets. These must be technically reviewed to ensure accuracy and acceptability for CODIS entry.
- 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.
- If multiple items have the same DNA profile, only one CODIS sheet should be used.
- DNA profiles or portions of DNA mixtures attributable to victims or elimination reference samples (boyfriend, husband, etc.) should not be entered into CODIS.
- CODIS sheets should be filled out for suspects that were not matched to an evidence sample or that were included as possible contributors to a DNA mixture.
- In general, CODIS sheets will be removed from the case file during the administrative review and given to the CODIS Administrator for entry.
- Searches of profiles in the local DNA database may be done prior to technical review of the case file.

Database Searches and Matches

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

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

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

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

Maintenance of a Staff Index in CODIS: Samples from laboratory employees and interns

Due to the sensitivity of the current DNA testing technology and its expanding role in the area of "touch" DNA evidence, all Criminalists, Forensic Specialists, Vault employees, and Interns in the Crime Scene and Forensic Biology Units must provide reference mouth swabs for inclusion of their DNA profile in the Staff Index of the local SDPD CODIS database. The following are the rules for the collection and analysis of these samples:

1. A SDPD Reference Mouth Swab Kit will be used to collect two swabs from the individual. Collection must occur as part of their orientation process. The Supervisor of the section involved has responsibility to both make sure the collection occurs and that appropriate protocol is followed.
2. All SDPD Reference Mouth Swab Kits from Staff and Interns will be permanently archived in the Forensic Biology Unit.
3. The Supervising Criminalist of Forensic Biology in conjunction with the DNA Technical Leader will arrange for the samples to be analyzed by a qualified DNA analyst.
4. The analysis of reference mouth swabs will follow the protocol used to analyze reference mouth swabs in criminal casework.

5. The DNA Technical Manager or CODIS Manager will enter the DNA profiles in the Staff Index of the local SDPD CODIS database.
6. The CODIS printout as well as the appropriately reviewed supporting documentation will be compiled and saved for each sample and included in the Staff DNA Profiles binder.

Reporting DNA results matching laboratory staff.

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

The CODIS match detail report indicating the match to a person in the staff database will be included in the analytical record.

If the DNA profile in the case was found to match (or the mixture includes) DNA from the analyst that worked on the case, then the report will state that DNA matching the analyst was found.

If the DNA profile in the case was found to match (or the mixture includes) DNA from a member of another section of the laboratory or another analyst in the Forensic Biology Unit, the report will state that a staff member of that section was found to match the DNA profile in the case. The report will also address whether any interpretations were affected by the presence of this DNA.

If the profile obtained from the evidence is a mixture and is still interpretable then the DNA profile from the staff member will be included in the table of results so that a qualified person could interpret the results as well.

A Staff Match Summary Sheet will be completed detailing the match and will include the name of the staff member. The summary will attempt to determine a likely cause for the presence of that DNA on the item (analyst from another section examined the evidence) and document any possible remediation to prevent future incidents.

The Staff Match Summary Sheet will be included in the log maintained in the Unit and will be available for review upon discovery request.

<u>Suggested wording of conclusions:</u>

Single source/predominant DNA matches staff member.

1. The DNA profile from Evidence Item X was found to match my DNA profile. No other item in this case contains this profile and therefore interpretations of these items were not affected.

2. The DNA profile from Evidence Item X was found to match DNA from a laboratory analyst from Unit X. No other item in this case contains this profile and therefore interpretations of these items were not affected.

Minor contributor scenario

1. …also detected in this sample was DNA that is consistent with my DNA profile. The presence of this DNA in the sample did not affect the overall interpretation for this item. No other item in this case contains this profile and the interpretations of these items were not affected.

…also detected in this sample was DNA that is consistent with a laboratory analyst from Unit X. The presence of this DNA in the sample did not affect the overall interpretation for this item. No other item in this case contains this profile and the interpretations of these items were not affected.

OUTSOURCING

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

- The vendor lab chosen 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 of the technical specifications of the analysis to be performed by the vendor laboratory is required.
- For any new outsourcing agreement an on-site visit to the vendor laboratory by the DNA Technical Manager, or another qualified analyst, is required.

ON-SITE VISITS TO VENDOR LABORATORIES

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

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

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

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

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

REVIEW OF OUTSOURCED DATA

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

- A review of the analytical record to include all controls (ladders, positive and negative amplification controls, and blanks.
- A review of the vendor laboratory's final report
- A review of the data for all submitted samples to the vendor laboratory.
- A verification of the DNA types and eligibility of any sample to be uploaded to the CODIS databases.

EQUIPMENT CALIBRATION/CHECK SCHEDULE

The following equipment has been identified as being critical to the analysis of samples in the Forensic Biology Section. The performance of this equipment will be assessed at approximately the intervals indicated.

Equipment Item	Location	Calibration Check
Pipettes	Forensic Biology Main Lab	Every Six Months
Balances	Forensic Biology Main Lab	Every three months
Pipettes	Amplification Room	Every Six months
Balances	Amplification Room	Every three months
BioRobot EZ1 - Volume Test	Forensic Biology Main Lab	Every four months
BioRobot Universal	Forensic Biology Main Lab	Annually
Thermal cyclers	Amplification Room	Every four months
Thermocouple	Amplification Room	Annually
7500 SDS: Well Contamination Check All other calibrations	Amplification Room	 Every four months Annually
Corbett CAS-1200	Amplification Room	Annually

MICROSCOPES

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

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^{o}C and freezers at a temperature colder than -10^{o}C. Deviations from the expected temperature indicate that maintenance or repair is likely required and the Supervising Criminalist of the unit will be notified.

PROCEDURE FOR THE CALIBRATION CHECK OF THE PIPETTES

The calibration of all pipettes in the Forensic Biology Laboratory is checked every six months. Two volumes within a pipette's range, given below, will be tested:

P2	0.5 and 1.5µL	E10	2.5 and 7.5µL
P10	2.5 and 7.5µL	E100	25 and 75µL
P20	5.0 and 15µL	E1000	250 and 750µL
P200	50 and 150µL		
P1000	250 and 750µL		

Water is used to measure the accuracy of the pipettes. The density of water at room temperature is 1g/mL, and, thus, 20µL of water weighs 20mg. Hence, when testing the accuracy of a P2 pipette, for example, 0.5µL of water should weigh out to 0.5mg on an analytical balance. There is a 5% tolerance limit for all pipettes, given below:

Pipette	Volume (µL)	Tolerance Limits (µL)
P1000	750	712.5 to 787.5
P1000	250	227.5 to 272.5
P200	150	142.5 to 157.5
P200	50	47.5 to 52.5
P20	15	14.25 to 15.75
P20	5	4.75 to 5.25
P10	7.5	7.125 to 7.875
P10	2.5	2.375 to 2.625
P2	1.5	1.425 to 1.575
P2	0.5	0.475 to 0.525

Pipette	Volume (µL)	Tolerance Limits (µL)
E1000	750	712.5 to 787.5
E1000	250	227.5 to 272.5
E100	75	71.2 to 78.7
E100	25	23.7 to 26.2
E10	7.5	7.12 to 7.87
E10	2.5	2.37 to 2.62

Pipettes that fall outside the tolerance limit will need to be sent to an outside company for recalibration. Once returned, the calibration will be verified by the Laboratory Technician before use in the laboratory.

To verify pipette calibration:

1. Place a piece of weighing paper on the analytical balance and tare the weight.
2. Set the pipette to the volume to be tested and draw that amount of water.
3. Carefully apply the water to the weighing paper.
4. Record the weight of the water.
5. Repeat for all pipettes at all test volumes.

PROCEDURE FOR THE CALIBRATION CHECK OF THE UNIT BALANCES

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

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

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

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

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

PROCEDURE FOR THE CALIBRATION CHECKS OF THE 9700 THERMOCYCLERS

The thermocyclers used for the polymerase chain reaction amplification of DNA are located in the Amplification Room of the Forensic Biology Unit. A calibration verification test and temperature non-uniformity test are conducted on each thermocycler every four months. The temperature non-uniformity test should be performed first. Records of the verification are kept in the Equipment Maintenance Log in the Amplification Room. Verification is conducted using a thermocoupler (a probe connected to a digital thermometer). The thermocoupler is sent to an outside company to be calibrated once each year.

Temperature non-uniformity test:
Eight wells will be tested for temperature uniformity by heating to two setpoints, 37ºC and 94ºC. Follow the instructions below, taken from the "Thermo Cycler Temperature Verification User's Manual" by Applied Biosystems pages 2-10 to 2-15.

1. Apply a few drops of mineral oil to a cotton swab and coat the following wells with oil: A1, A12, C4, C9, F4, F9, H1, and H12.

2. Place the 9700 Temperature Verification Frame on the sample block.

3. Connect the probe to the digital thermometer and turn the instrument on.

4. Place the probe in well A1, thread the probe wire through the channel in the Verification Frame, slide the heated cover forward and pull the lever down.

5. Turn on the system 9700 and the **Main** menu appears.

6. Press **F4** (Util) and the **Utilities** screen appears.

7. Press **F1** (Diag) and the **Diagnostics** screen appears.

8. Press **F3** (TempVer) and the **Temperature Verification** screen appears.

9. Press **F2** (TNU). This automatically configures the system 9700 for the Temperature Non-Uniformity Test, starting with the setpoint of 37ºC. The **TNU Performance** screen appears.

10. Press **F1** (Run). This starts the Temperature Non-Uniformity Test. The **TNU Performance** screen appears with the setpoint value displayed.

11. Note: The sample block must be within 1.0ºC of the setpoint. In addition, the cover must be within 1ºC of 35ºC. It may take several minutes for the system 9700 to stabilize at the setpoint temperature.

12. The **TNU Performance** screen counts down the time until the setpoint is stabilized. When the "Stabilizing at setpoint" value decrements to zero, read the digital thermometer.

13. Note: The thermocoupler will periodically shut itself off to save power. Simply press the power button and it will read the current temperature of the well.

14. Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field. Record this value on the Temperature Non-Uniformity Log.

15. Note: The digital thermometer displays a four-digit value; round the value off to three digits before typing it in the **TNU Performance** screen.

16. Press **Enter**. Slide the heated cover back and move the probe to well A12.

17. Repeat steps 10-13 until a temperature reading is recorded for the remaining wells at the setpoint of 37ºC.

18. Press **Enter**. The system 9700 automatically begins the second reading at 94ºC setpoint. The **TNU Performance** screen appears with the setpoint value displayed.

19. Note: The sample block must be with 1.0ºC of the setpoint. In addition, the cover must be with 1ºC of 105ºC. It may take several minutes for the system 9700 to stabilize the setpoint temperature.

20. Place the probe in well A1 and repeat steps 10-13 for the second setpoint for the remaining wells.

21. The system 9700 evaluates the uniformity of the sample block temperature for the setpoint values you entered and displays the results. A summary screen appears at the conclusion of the test.

22. Press **F1** (Accept). Record the given TNU Performance results in Temperature Non-Uniformity Log.

23. Press **F5** (Exit).

24. Continue on to the Temperature Verification test or, if finished, remove the probe and clean the sample block using reagent alcohol.

25. Turn off the system 9700.

Temperature Verification:
Two temperature setpoints, 85ºC and 45ºC, are verified in one well, A6. Follow the instructions below, taken from the "Thermo Cycler Temperature Verification User's Manual" by Applied Biosystems pages 2-3 to 2-9.

1. Use a cotton swab to coat well A6 with mineral oil.

2. Place the 9700 Temperature Verification Frame on the sample block.

3. Connect the probe to the digital thermometer and turn the instrument on.

4. Place the probe in well A6, thread the probe wire through the channel in the Verification Frame, slide the heated cover forward and pull the lever down.

5. Turn on the system 9700 and the **Main** menu appears.

6. Press **F4** (Util) and the **Utilities** screen appears.

7. Press **F1** (Diag) and the **Diagnostics** screen appears.

8. Press **F3** (TmpVer) and the **Temperature Verification** screen appears.

9. Press **F1** (Temp). This automatically configures the system 9700 for the Calibration Verification Test. The **Calibration Verification** screen appears.

10. Press **F1** (Run). This starts the Calibration Verification Test. The **Calibration Verification** screen appears with the setpoint value (85ºC) displayed.
    Note: the cover must be within 1ºC of 105ºC, It may take several minutes for the system 9700 to ramp up.

11. The **Calibration Verification** screen counts down the time until the setpoint is reached. When the "Stabilizing at setpoint" value decrements to zero, read the digital thermometer.

12. Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field. Record this value in the Temperature Verification Log.

Note: The digital thermometer displays a four-digit value; round this number off to three digits before typing it in the **Calibration Verification** screen.

13. Press **Enter**. The system automatically begins the second reading (45ºC setpoint). The **Calibration Verification** screen appears with the setpoint value displayed.

Note: The cover must be within 1ºC of 105ºC.

14. Repeat steps 11-13 for the second reading.

15. The system 9700 evaluates the calibration of the sample block temperature for the setpoint values you entered and displays the result. A summary screen appears at the conclusion of the test.

16. Press **F1** (Accept).

17. If the sample block module is properly calibrated, then the **Calibration Verification** screen appears with the message "Calibration is good." If the sample block module does not pass the Calibration Verification Test the **Calibration Verification** screen appears with the message "Instrument may require service. Contact Applied Biosystems Technical Support."

    a. If the test fails, repeat the procedure to make sure the thermocoupler was not misread or that errors were not made when entering data.
    b. If the test fails again, contact Applied Biosystems Technical Support.

18. Press **F5** (Exit).

19. Clean the sample block of mineral oil using reagent alcohol.

20. Turn off the system 9700.

Method: **Block Contamination Check on the SDS 7500 Instruments**

Date: 11/15/06 Revision Date: 03/01/2010 Approved by: SAM

Purpose:

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

Preparing the Check

This check is performed without a plate being positioned in the plate holder; therefore, if there is a plate in the holder, remove it, and then push the carriage forward to close it.

Note: This check is for information only and should not be saved during or after the check.

Performing the Check

1. Turn on the instrument and open the SDS software and select calibrate from the Instrument drop-down menu (**Instrument>Calibrate)**.

2. Using the Filter A (FAM filter position), open the ROI Inspector and take an image at 2048ms by selecting **Snapshot**.

3. Observe the background fluorescence in the 96 wells. Note any wells that have significant fluorescence; fluorescence indicates contamination. For documentation, perform a Screen Shot (**Alt + PrintScrn)** and then paste it (**Ctrl-V)** into a Microsoft Word blank document.

4. To determine an acceptable background fluorescence level:
   a. Push the instrument carriage and allow it to spring back to open it.
   b. Put a new, clean, empty reaction plate without the cover into the sample block.
   c. Pull the carriage forward and pull the door down to close it.
   d. Take another image at 2048 ms. For documentation, perform a Screen Shot (**Alt +PrintScrn)** and then paste it (**Ctrl-V)** into a Microsoft Word blank document.
   e. Save as Calibration Contamination Check(MM/DD/YY) in the appropriate instrument folder.

5. If some of the wells still appear to have significant fluorescence, clean them per the instructions in the “Cleaning the Sample Wells” section of the “Decontaminating the Sample Block” section of Appendix A in the 7500 maintenance guide.

Recheck the wells after cleaning, following steps 1 through 4 above.

REFERENCE:

ABI Prism 7000 Sequence Detection System User Guide.

Method: **Preparing the ROI (Regions of Interest) Calibration Plate for the 7500 SDS Instrument**

Date: 11/15/2006 Approved by: SAM

MATERIALS REQUIRED:

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

Purpose:

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

Preparing the Plate

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

Creating a Plate Document for the ROI Calibration

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

b. In the Sat Threshold field, enter **4000**.
c. Click **OK**

6. In the ROI Inspector dialog box, set the lamp control:
   a. Click **Block up**
   b. Select **Idle** from the Lamp Control drop-down list

Performing the ROI Calibration

1. In the ROI Inspector dialog box:
   a. In the Exposure Time field, enter **256** for the 7000 and **2048** for the 7500 and
   b. Select **Filter A.**

2. Click **Snapshot** to generate an ROI image.

3. Determine if your ROI image is acceptable. An acceptable image will not have saturated red areas in any wells. (A few red pixels in a well are acceptable).

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

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

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

5. Click **Save Calibration** to save the image for filter A. (An OK will appear in the dialog box for Filer A). Remember to **Save** after every filter.

6. Repeats steps 1 through 6 for the remaining filters: Filters B, C, D and E. Reset the Exposure Time to 256 or 2048 before performing the calibration for each filter.

7. Click **Done > File > Close** and click **NO** to save the plate document.

Unloading the Plate

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

REFERENCE:

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

Method: **Performing the Background Calibration on the 7500 SDS Instrument**

Date: 11/15/06 Revision: 02-20-2009 Approved by: SAM

MATERIALS REQUIRED:

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

Purpose:

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

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

Precautions:

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

Preparing the Background Calibration Plate

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

Creating a Plate Document for the Background Calibration

1. Place the prepared plate into the plate holder and close the tray/door. Start the instrument and open the SDS software. Click **File > New**.
2. Configure the New Document Dialog box:
   a. Select **Assay >Background**.
   b. Select **Container > 96-Well Clear**.

c. Select **Template > Blank Document**
d. In the Operator field, enter a name.
e. In the plate Name field, enter : **Background_<date *MMDDYY*>** and click **Finish**.

3. In the SDS software select **File > Save As**. (Navigate to D\: > AppliedBiosystems >SDS Documents and Save). Select **Save as type > SDS Documents (*.sds)**.

Performing the Background Calibration

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

Analyzing the Background Calibration Data

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

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

2. In the plate document, select the **Results** tab, then **Spectra** tab. Print this page for the Background Calibration records and to show that the instrument is free of significant contaminants.

3. Inspect the raw data for irregular spectral peaks that exceed the following fluorescent standard units (FSU). All wells should be selected.

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

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

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

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

5. Select **File > Close**

REFERENCE

1. Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

Method: **Performing the Optical Calibration for the 7500 SDS Instrument**

Date: 11/15/2006 Approved by: SAM

MATERIALS REQUIRED:

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

Purpose:

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

Preparing the Background Calibration Plate

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

Creating a Plate Document for the Background Calibration

1. Start the instrument and open the SDS software. Load the ROI calibration plate into the plate holder position.
2. Configure the New Document Dialog box:
   a. Select **Assay >Calibration**.
   b. Select **Container > 96-Well Clear**.
   c. Select **Template > Blank Document**
   d. In the Operator field, enter a name.
   e. In the plate Name field, enter :
   Calibration_<**date *MMDDYY***> and click **Finish**.
3. In the SDS software select **File > Save As**. (Navigate to D\:>AppliedBiosystems>SDS Documents and Save)

Performing the Background Calibration

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

Analyzing the Optical Calibration Data

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

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

REFERENCE:

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

Method: **Performing the Pure Dye Calibration on the 7500 SDS Instrument**

Date: 12/02/2008 Revision: 02-20-2009 Approved by: SAM

MATERIALS REQUIRED:

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

2. Centrifuge and vortex

Purpose:

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

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

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

Preparing the Plate

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

Creating a Plate Document for the Pure Dye Calibration

1. Start the instrument and open the SDS software. Click **File > New.**

2. In the New Document Wizard dialog box:

    a. Select **Assay > Pure Spectra.**

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

Selecting the Dye

1. In the Pure Spectra Calibration Manager dialog box:

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

Preparing and Loading a Pure Dye Plate

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

Performing the Pure Dye Calibration

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

Calibrating remaining dyes:

1. Click **Next Dye >.**
2. Repeat the procedures in Preparing and Loading a Pure Dye Plate and Performing the Pure Dye Calibration to run the remaining pure dye plates.

3. After you calibrate the instrument with all pure dyes provided in the spectral calibration kits, click **Finish.**

Analyzing the Pure Dye Calibration Data

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

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

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

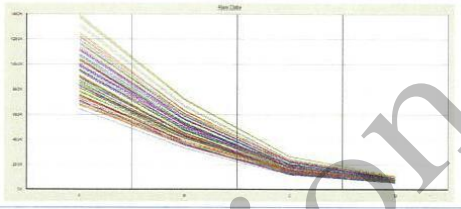
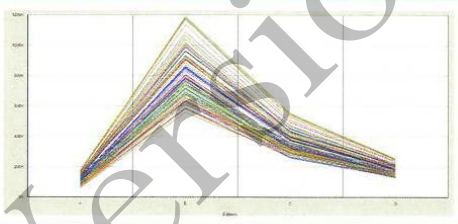
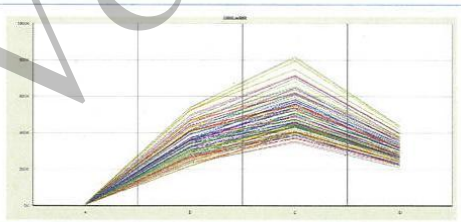
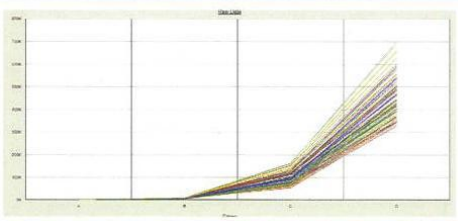
5. In the pure dye plate document:

   a. Select the **Results** tab.
   b. Select the **Spectra** tab.

6. Select all wells of the plate document by clicking the upper-left corner of the plate grid.

7. Using the tables below as a reference, verify that the peak for the spectrum of the pure dye occurs at the correct filter. For documentation, **Print** the spectra for each dye (**Alt + Print Scrn**; then open a new blank Microsoft Word document

and **Ctrl V** to paste the spectra). If the peak for the spectra of a dye occurs in the wrong filter, you may have run the wrong dye plate during the calibration. Repeat the procedure using the correct dye.

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

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

9. Repeat steps 1 through 8 to extract the calibration data for the remaining pure dyes.

REFERENCE:

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

Method: **Running the TaqMan RNase P Plate Data on the ABI 7500 SDS**

Date: 11/15/2006 Revision: 02/20/2009 Approved by: SAM

MATERIALS REQUIRED:

1. Applied Biosystems Real-Time PCR System Spectral Calibration Kit
2. TaqMan RNase P Instrument Verification Plate (Part No. 4350584)
3. Centrifuge and vortex

Purpose:

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

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

Preparing the TaqMan RNase P Plate

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

Creating a Plate Document for the RNase P Verification Run

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

e. In the SDS software, select **File> Save As.** (Navigate to D\:>AppliedBiosystems>SDS Documents and Save)

Performing the Verification Run

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

Analyzing the TaqMan RNase P Plate Data

1. Select **Analysis > Analysis Settings**. Select **Auto C_t** >Click **OK.** Click the green arrow or select **Analyze.**

2. Assess the results. In the plate document: Select the **Results** tab then select the **Amplification Plot** tab.

1. Display the data: from the Amplification Plot tab:

   a. Click the upper-left corner of the plate grid to select all wells.

   b. Select **Data > C_t vs. Well Position** to display the plots. Print this data.

2. Verify the uniformity of each replicate population by comparing the groupings of C_t values.

If outliers are present, omit and reanalyze (*see page 93 of the 7500 Installation and Maintenance Guide for trouble shooting, this will apply to the 7000, as well).*

3. In the results tab, select the **Standard Curve** tab and verify that the R^2 value is $\geq$ 0.990.

4. In the **Report** tab, calculate the verification value of the 5,000-copy and 10,000-copy populations:

   a. For the 5,000-copy populations, in the Report tab, scroll to a sample in the 5K population. Apply the value in the Mean Qty and StdDev Qty columns to the following equation:

      5K value = Mean Qty + 3(StdDev Qty)

   b. For the 10,000-copy populations, in the Report tab, scroll to a sample in the 10K population. Apply the value in the Mean Qty and StdDev Qty columns to the following equation:

10K value = Mean Qty – 3(StdDev Qty)

5. Compare the values of the 10K and 5K-copy populations. If the 10K value is greater than the 5K value the 7500 has passed.

6. **With all wells chosen, print the Report.** Save the Tabular Report Data and the Standard Curve Data pages. You may discard the Raw Data and the Delta Rn vs Cycle Data pages. **It is recommended that the calculations performed above be written on one of the Tabular Report Data pages.**

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

REFERENCE:

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

Method: **Monitoring and Replacing the Lamp on the 7500 SDS Instrument**

Date: 02/20/2009 Approved by: SAM

Monitoring the Lamp Status

The halogen lamp has a life span of approximately 1,000 hours.

1. Select **File > New.**

2. In the New Document Wizard, click **Finish.**

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

   Good—the lamp is functioning well. There is no need to replace the lamp bulb at this time. Click **Close.**
   Failed—The lamp bulb must be replaced. Click **Close**, then replace the lamp.
   Change Soon—the lamp bulb usage is above 2000 hours. It is recommended to replace the lamp soon. Click **Close**, then replace the lamp.

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

Replacing the Halogen Lamp on the 7500 SDS Instrument

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

MATERIALS REQUIRED:

1. Halogen bulb (12V, 75W)

2. Small pointed object, for example a screwdriver.

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

1. Power off, then unplug the 7500 SDS Instrument. Allow the instrument to cool for 15 minutes.

2. Open the access door to the Instrument:
   Insert a thin screwdriver or other object into the keyhole on the edge of the front access door, then push to unlatch the door. Open the door.

3. Remove the lamp from the instrument:
   Slide the lamp release lever downward. Firmly grasp the lamp and lift it up and out of the slotted mount.

4. Inspect the lamp for signs of failure (see the Installation and Maintenance Guide page 124 for indications of lamp failure).

5. Place the new lamp into the instrument
   Slide the lamp release lever upward. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.

6. Close the access door.

7. Plug in and power on the 7500 SDS Instrument.

8. Open the ROI Inspector dialog box
   If the Quick Startup document dialog box is open, select **Create New Document**. If the Quick Startup document dialog box is not open, select **File > New**. In the New Document wizard, click **Finish**. In the SDS software, select **Instrument > Calibrate**.

9. In the ROI Inspector dialog box, select **Lamp Control > Idle.**

10. While the instrument is running, look through the grating of the access door and verify that the lamp is illuminated, then click **Done**.

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

<u>REFERENCE:</u>

Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide.

OPERATION OF THE pH METER

The pH meter is calibrated before each use. The Orion 420A meter is able to automatically recognize 4.00, 7.00, and 10.00 buffers with a range of ± 0.05 pH units. During calibration, the user waits for a stable pH reading. Once the electrode is stable the meter automatically recognizes and displays the temperature corrected pH value of that buffer. Pressing **yes** enters the value into memory.

To calibrate the pH meter, use two buffers with pH values that bracket the expected pH of the sample being tested (for example, when measuring a sample whose pH is expected to be 8, use buffers with pH 7.00 and pH 10.00), and follow the directions below:

1. Press the **mode** key until the pH mode indicator is displayed.

2. Press **2nd CAL**. CALIBRATE is displayed above the main field. The time and date of the last calibration are displayed. After a few seconds, P1 is displayed in the lower field indicating the meter is ready for the first buffer.

3. Rinse the electrodes with distilled water and place it in the first buffer.

4. When the electrode is stable the READY prompt will be displayed and the temperature corrected value for the buffer is displayed. Press **yes**.

5. The display will remain frozen for a few seconds then P2 will be displayed in the lower field indicating the meter is ready for the second buffer.

6. Rinse the electrodes with distilled water and place it into the second buffer.

7. Wait for a stable pH display and press **yes**.

8. After the second buffer value has been entered the electrode slope will displayed.

9. SLP appears in the lower field while the actual electrode slope in percent appears in the main field.

10. The meter automatically advances to the measure mode, and MEASURE is displayed above the main field.

11. Rinse the electrodes with distilled water and place into your sample.

12. Record the pH directly from the main meter display.

13. Record these values in the pH Meter Calibration Log in the Equipment Maintenance Log.

14. Rinse the electrodes with distilled water and replace in electrode storage solution. The solution should fill the electrode to within 1/4 inch of the filling hole.

Note: The electrode currently in use in the pH meter in the Forensic Biology Unit should be filled with 4M KCl saturated with AgCl (Orion part# 900011 or Fisher# SP-135. Caution! Different electrodes need different filling solutions; not all the solutions are interchangeable. Adding the wrong filling solution can damage or destroy an electrode. Make sure you have the right filling solution before adding it to an electrode.

OPERATION AND MAINTENANCE OF THE WATER PURIFICATION SYSTEM

There are two components to the water purification system: distilled water and ultra/nano pure water. The operation of the two components is independent, although the ultra/nano pure system uses the distilled water reservoir as its water source.

Distilled water
City tap water is distilled by passing through a de-mineralizing filter tank and is subsequently boiled. The condensation is then collected in a 100 liter carboy, from which the now distilled water is dispensed for use in the laboratory. Every four months the 100 liter carboy is cleaned.

Operating the distilled water system:
The water level in the carboy can be checked by holding up the tube and opening the spigot. The water will fill the tube to the level in the carboy. Be sure that the D-45 de-mineralizing filter tank indicator light is on. If the red light is out, the tank must be replaced. An outside company will change the tanks.

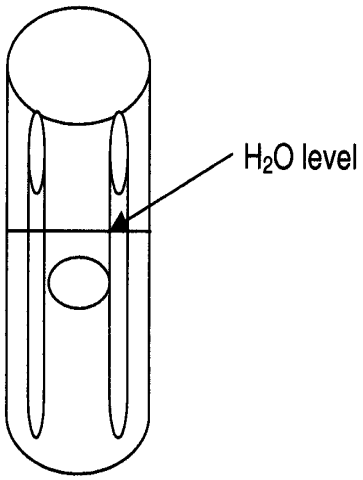
1. Flip the switch on the filter tank to the vertical position. This turns on the overflow for the filter.

2. Turn the system on by pushing the 'POWER ON' button.

3. Turn on the heaters by pushing the 'HEATERS ON' button.

Make sure the water level within the heating condenser is above the white oval. Check the cooling coil and make sure no bubbles are present. To dispense water, flip the handle on the spigot counter clockwise. To switch the system off, simply turn of the power, heaters, and close the overflow switch. The carboy has a water level sensor, which will shut off the power when the reservoir is full.

Diagram:



Changing filter tanks:
The filter tanks have a red indicator light to show when a tank needs to be changed. Two tanks are kept at a time, and when both have been used, an outside company (currently Siemens Water Technologies, 858-391-5555) will be called to replace the tanks.

1. Unplug the Barnstead FiStreem II distilled water system.

2. Unplug the indicator light for the filter tanks.

3. Shut off the water valve.

4. Drain the water remaining in the distilled water system (not the carboy) by turning the knob on the side of the system to the open position.

5. Undo the tubing connected to the filter tank and replace it on the new tank.

6. Plug everything back in and turn on the water.

7. Turn on the water distilling system, but not the heaters, and adjust the water flowing into the system.

8. Once the water flow into the system has been adjusted, the system may be run as normal.

It may take a while for the indicator light to come on; as the water needs to pass through the whole filter for the light to come on.

Cleaning the distilled water carboy:
The 100 liter carboy containing distilled water is cleaned every four months.

1. Fill the ultra/nano pure water carboy with the water remaining in the distilled water carboy. This empties the distilled carboy so it can be cleaned and the collected ultra/nano pure water will be used to rinse the distilled carboy.

2. Use protective gloves, safety glasses, and a lab coat when cleaning the carboy.

3. Turn off the water valve.

4. Turn off and unplug the Barnstead FiStreem II distilled water system.

5. Turn off and unplug the Barnstead NANOpure ultra pure water system.

6. Unplug the conductivity wire connected to the D-45 filter tank.

7. Carefully unscrew the grey shut-off valve.

8. Carefully remove all tubing and anything else connected to the carboy, including the spigot.

9. Remove the metal casing enclosing the 100 liter carboy.

10. Wash the carboy three times with warm tap water and Liquinox detergent. Use the extended-handle brush to scrub inside the carboy.

11. Rinse the carboy with tap water at least three times to remove the detergent.

12. Rinse the carboy three times with ultra/nano pure water.

13. Dry off carboy and clean off metal casing.

14. Carefully reassemble all components of the water purification system and tighten everything to prevent leaks.

15. Record the cleaning on the Carboy Maintenance Log in the Equipment Maintenance Log.

Ultra pure water

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

MAINTENANCE OF THE QIAGEN BIOROBOT EZ1s

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

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 u**. 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.

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.

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.

12. 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.

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.

GENERAL LABORATORY MAINTENANCE

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

- Monitor the operating temperatures of all freezers, refrigerators, and incubators. Log temperatures on the log sheets. When a sheet has been completed, turn it in to the Supervising Criminalist of the Forensic Biology Unit.
- Wipe general use counters with 10% bleach solution.
- Clean common use centrifuges with 10% bleach solution followed by wiping with distilled water.
- Wipe interior of extractions hoods with 10% bleach solution (do not wipe plastic door).
- Clean interior and exterior of drying boxes with 10% bleach solution.
- Wash glassware and dishes not belonging to an analyst (analysts are responsible for their own dishes). Return washed dishes to their appropriate storage location.
- Fill distilled water carboys, reagent alcohol bottles, 10% bleach solution bottles, and Liquinox detergent bottles.

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

<u>Glassware washing protocol:</u>
It is the responsibility of each analyst to wash their own dishes. Protective gloves, safety glasses, and a lab coat or apron should be worn.

1. Wash glassware in warm water with Liquinox detergent.
2. Rinse detergent from glassware by rinsing in tap water three times.
3. Rinse each piece of glassware in distilled water three times.
4. Set glassware to dry.

5. Return glassware to appropriate storage location when dry.

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

CLEANING LOGS

The following cleaning logs will be maintained by the Forensic Biology Unit. They are intended to ensure common use areas are kept in a clean state.

Cleaning Log	Location	Updated
Forensic Biology Main Lab	Forensic Biology Main Lab	Weekly
Amplification Room	Amplification Room	Weekly/ Biweekly (floors)
Instrument Washroom	Instrument Washroom	Weekly
Evidence Evaluation Room	Evidence Evaluation Room	Weekly
Water purification carboy	Forensic Biology Main Lab	Every four months
Qiagen BioRobot® EZ1	Forensic Biology Main Lab	Monthly
Qiagen Universal	Forensic Biology Main Lab	As used

CLEANING AND DECONTAMINATION PROCEDURES

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

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

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

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

For items that cannot be autoclaved, they will be treated first with bleach as previously described and then autoclaved for thirty minutes.

Decontamination of centrifuges

Fisher "Marathon" rotors - clean the top surface of the rotor with detergent, and rinse with distilled water. For Model 16 K/M, the rotor can be removed from the spindle for cleaning.

Sorvall A384 fixed angle rotor - use clean tubes and clean tubes with soap and water after each use. Rinse thoroughly with distilled water and dry. Periodically, inspect the surface of the rotor body and cover for signs of contamination. The rotors can be removed and washed in a pan of soapy water (use Liquinox detergent). If a serious contamination problem occurs (ie spilled concentrated DNA), the rotor body, cover, and tube holders can be autoclaved.

Sorvall H1000B swinging-bucket rotor - clean the rotor buckets and tube holders with detergent. Rinse thoroughly with distilled water and dry. Periodically, inspect the rotor body for signs of contamination. The rotors can be removed and washed in a pan of soapy water (use Liquinox detergent). If a serious contamination problem occurs (ie spilled concentrated DNA), the rotor body, cover, and tube holders can be autoclaved.

DISPOSAL OF HAZARDOUS WASTE

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

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

Phenolphthalin and Leucomalachite Green (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.

EZ1 reagent waste
EZ1 strips are flammable because of the ethanol they contain and should be placed in the blue 30gal drum by the instruments. All tips, tip holders, and tubes can be disposed of as regular trash. When the 30gal drum is full the waste can be transferred to the metal 50gal drum in the Chemical Storeroom for disposal.

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

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.

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.

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

PREPARATION OF EVIDENCE COLLECTION KITS

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

The Laboratory Technician is responsible for preparing and disseminating the 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.

Reference Mouth Swab Collection Kits:
Keep 50-60 kits on hand at all times.

Place the following items in a Reference Mouth Swab Collection Kit envelope (a manila envelope with an 'SDPD REFERENCE MOUTH SWAB COLLECTION KIT' sticker and a red text 'SDPD REFERENCE MOUTH SWABS' information sticker):

- 2 sterile, paper-wrapped swabs
- 1 manila envelope with mouth reference swabs information label in red text
- 1 smaller manila envelope
- 1 evidence seal
- 1 pair of large latex gloves
- 1 copy of 'Instructions for the Collection of Reference Mouth Swabs'

Individual kits can be packaged together in large paper bags containing 10, 15, 20, etc kits.

Biological Stain Collection Kit:
Keep 40-50 kits on hand at all times.

Place the following items in a Biological Stain Collection Kit envelope (a manila envelope with an 'SDPD BIOLOGICAL STAIN COLLECTION KIT' sticker and a black text 'SDPD EVIDENCE SAMPLE' information sticker):

- 2 sterile, paper-wrapped swabs
- 1 tube containing sterile water
- 1 manila envelope with biological stain information label
- 1 smaller manila envelope
- 1 evidence seal
- 1 pair of large latex gloves
- 1 copy of 'Instructions for the Collection of a Biological Stain'

Victim Sexual Assault Examination Kit:
Keep 60-80 kits on hand at all times.

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

- 5 swube tubes
- 1 Pubic Hair Combings envelope with comb
- 1 Head Hair envelope with bindle
- 1 Pubic Hair envelope with bindle
- 1 Left Fingernail Scrapings envelope with 2 toothpicks in a bindle
- 1 Right Fingernail Scrapings envelope with 2 toothpicks in a bindle
- 1 piece of tissue paper
- 1 evidence seal
- 1 victim label sheet (1 Cervical Swabs, 1 Vaginal Swabs, 1 External Genital Swabs, 1 Oral swabs, and 1 Rectal Swabs sticker)
- 2 sterile, paper-wrapped swabs
- 1 manila envelope with mouth reference swabs information label in red text

Suspect Sexual Assault Examination Kit:
Keep 40-60 kits on hand at all times.

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

- 3 swube tubes
- 1 Pubic Hair Combings envelope with comb
- 1 Head Hair envelope with bindle
- 1 Pubic Hair envelope with bindle
- 1 Left Fingernail Scrapings envelope with 2 toothpicks in a bindle
- 1 Right Fingernail Scrapings envelope with 2 toothpicks in a bindle

- 1 piece of tissue paper
- 1 evidence seal
- 1 suspect label sheet (2 Penile Swabs, 2 Scrotal Swabs, and 2 Oral swabs stickers)
- 2 sterile, paper-wrapped swabs
- 1 manila envelope with mouth reference swabs information label in red text

Physiological Samples Kit:
Keep 60-80 kits on hand at all times.

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

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

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

Fetal Tissue Collection Kit:
Keep 1-5 kits on hand at all times.

Place the following items in a manila envelope:

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

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

ORDERING AND RECEIVING COMMERCIAL SUPPLIES

Ordering procedure

Requests for supplies, reagents, and DNA typing kits will be placed in the Supply Order Log located in the Forensic Biology Unit.

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

Receiving supplies

The date, initials of the person receiving the supplies, and the location where the supplies will be stored will be logged into the Supply Order Log.

Receiving chemicals

When receiving chemicals, the same procedure as receiving supplies should be followed except the lot number must be recorded in the Supply Order Log.

The chemical name/description, lot number, and other pertinent information will be recorded on the crime laboratory's chemical database, by the receiver.

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

The date a chemical is opened for first use and by whom must also appear on the container.

Receiving DNA kits

The kits used for DNA extraction, quantification, and typing (including bulk Taq DNA polymerase) must all be recorded in the Kit Receipt Log upon receipt. In general, the lot numbers of every component of the kit are recorded as well as the lot number of the kit. Kit components may be stored in different locations and, thus, all kits components must be labeled with the lot number of the whole kit. Some kits will require a quality control test before use.

<u>Qiagen DNA Investigator Kits</u>
Record lot numbers of all kit components, except the proteinase K, and the whole kit in the Kit Receipt Log. Discard the proteinase K. This kit must be quality control tested before use. 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.

Quantifiler kits
Record lot numbers of the PCR reaction mix, DNA standard, primer, and whole kit in the Kit Receipt Log. The PCR reaction mix should be labeled with the whole kit lot number and stored in the reagent refrigerator. The remainder of the kit (in the original box) will be stored in the reagent freezer. This kit must be quality control tested before use. 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.

Identifiler
Record lot numbers of all components of the kit and the whole kit lot number in the Kit Receipt Log. The PCR reagents (blue box) should be labeled with the whole kit lot number and stored in the reagent refrigerator. The AmpliTaq should be placed in a freezer holder with tubes numbered (1 thru X), labeled with the whole kit lot number and stored in the reagent freezer. The ladder should be labeled with the whole kit lot number and stored in the reagent refrigerator in the Amplification Room. This kit must be quality control tested before use. 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.

Yfiler
Record lot numbers of the whole kit and kit components in the Kit Receipt Log. The PCR reagents should be labeled with the whole kit lot number and stored in the reagent refrigerator. The AmpliTaq should be placed in a freezer holder with tubes numbered (1 thru X), labeled with the whole kit lot number and stored in the reagent freezer. The allelic ladder should be labeled with the whole kit lot number and stored in the Amplification Room reagent freezer. This kit requires quality control testing before use. Place "Not QC'd, Do Not Use" stickers on the kits until quality control testing has been completed. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

Minifiler
Record lot numbers of the whole kit and kit components in the Kit Receipt Log. The PCR reagents should be labeled with the whole kit lot number and stored in the reagent refrigerator. The allelic ladder should be labeled with the whole kit lot number and stored in the Amplification Room reagent freezer. This kit requires quality control testing before use. Place "Not QC'd, Do Not Use" stickers on the kits until quality control testing has been completed. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.

Bulk Taq DNA Polymerase
Record lot numbers of the whole kit and kit components in the Kit Receipt Log. The AmpliTaq should be placed in a freezer holder with tubes numbered (1 thru X), labeled "Bulk Taq" and stored in the reagent freezer. Place "Not QC'd, Do Not Use" stickers on the kits until quality control testing has been completed. Replace with "QC OK" stickers once the quality control tests have been completed and reviewed.