Exogenous DNA Prevention

GUIDING PRINCIPLES

Exogenous DNA is defined as the addition of DNA/biological fluid to evidence or controls subsequent to the crime. Sources of exogenous DNA could be first responders, crime scene technicians, NYPD personnel, or laboratory personnel, to name a few.

It is the goal of the Department of Forensic Biology to not transfer any DNA from employees to any casework sample. Several measures have been taken to prevent this, and this document will cover these measures in general.

PROCEDURE

A. Facility

The laboratory is divided into physically isolated areas for evidence examination, DNA extraction, pre-amplification (amplification setup) and post-amplification (amplification and DNA typing). Each area has its own dedicated equipment. Once samples are accepted into the laboratory, they move through these areas in one direction only. Samples are first processed in the evidence examination area. They are then moved to the DNA extraction area. Following DNA extraction, aliquots of each sample are quantitated in the DNA quantitation area. Following DNA quantitation, aliquots of each sample are moved into the pre-amplification area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and typed in the post-amplification area. This laboratory setup helps eliminate the travel of DNA from post-amplification areas back into non-amplified DNA areas.

B. Laboratory Clean-up

In addition to the separation of space between analyses, the Department has implemented a documented clean-up program on a monthly basis. The documented clean-up program may be more frequent in areas where High Sensitivity DNA Testing is performed. The clean-up program involves the decontamination of instruments/equipment, bench/counter tops, sinks, etc. While 10% Bleach is extremely effective in destroying exogenous DNA, it is also very corrosive. Care should be taken so that when 10% Bleach is used, it is immediately followed by 70% Ethanol and/or water to wash off the Bleach from the surface of instruments/equipment.

C. Sample Processing

Exemplar samples are processed separately from evidence samples. Also, only one sample is processed at a time using single-use disposable supplies whenever possible (e.g,. pipette tips), and scissors/tweezers are thoroughly cleaned between each sample.

D. Personal Protective Equipment (PPE)

PPE is designed to protect employees from serious workplace injuries or illnesses resulting from contact with chemical, reagents, or biological hazards. PPE includes a variety of devices and garments such as goggles, gloves, lab coats, etc. Proper PPE must be worn during analysis, and required PPE may vary from location to location depending on the hazards of the area. While PPE is designed to protect employees, it can also prevent the transfer of DNA from employees to work surfaces or evidence.

E. Contamination Prevention Equipment (CPE)

CPE is designed to prevent the occurrence of exogenous DNA in samples. While all PPE are considered as CPE, not all CPE can be considered as PPE. For example, in clean-rooms of the laboratory where high sensitivity DNA testing takes place, the wearing of booties or bouffant caps is to prevent the transfer of DNA from employees. CPE must be worn when designated and available. If not available, employees must first seek permission to work in that area from the appropriate Technical Leader and exercise extreme caution to maintain a clean environment.

F. Identification

Exogenous DNA may be indicated by 1) the presence of signal in reagent blanks, 2) the presence of extraneous alleles in positive controls, or 3) the presence of extraneous alleles in case samples. The confirmation of exogenous DNA may reflect a system failure or contamination of the samples by an outside source. The source may be equipment, reagents, the working environment, laboratory/law enforcement personnel, or an analytical error. It can either be a single isolated event (such as cross-contamination between two samples) or it can be persistent (such as dirty reagents or equipment). To remedy a single isolated event, the appropriate extraction, quantitation, amplification and/or STR analysis is repeated.

To aid in the identification of exogenous DNA, the LAB TYPES DATABASE procedure is used.

The Quality Assurance Manager and/or the appropriate Technical Leader must be notified if exogenous DNA is detected. The source of this DNA should be identified, if possible, and eliminated. For persistent events, the QUALITY INCIDENT REVIEW procedure must be followed to prevent the recurrence of the problem.

G. Interpretation and reporting

Samples containing exogenous DNA must be interpreted and reported carefully. This is further discussed in the GENERAL GUIDELINES FOR DNA CASEWORK procedure.

Revision History:

February 9, 2010 – Initial version of procedure.

Lab Types Database

GUIDING PRINCIPLES AND SCOPE

"Lab Types" is a DNA database that contains the DNA profiles of individuals who have access to laboratory space and/or may come into contact with an item of evidence prior to or during processing. It contains locally- and nationally-recognized exogenous DNA profiles. This database is a part of the Quality Assurance Program of the laboratory and must be searched in order to assure that no casework DNA profile was contributed by someone during or after the investigation.

The individuals included in Lab Types include past and present personnel of the OCME, members of housekeeping staff, equipment vendors, select members of NYPD, and various visitors to the laboratory. Any DNA profiles that link cases together but are found to be exogenous will be kept in Lab Types under a contaminant listing.

This procedure describes the collection, identification, processing, and disposition of samples used to create the DNA profiles stored in the database. It also describes the processes for the operation and maintenance of the database as well as how the database is used by casework analysts.

PROCEDURE

A. Sample Collection

1. All samples collected internally for Lab Types processing must be collected by an authorized individual. The OCME Human Resource Department most often collects and records each swab taken. Swabs are then sent to Forensic Biology for Processing.

2. The proper consent form must be completed by the donor prior to the collection of the swabs. This form will be stored with the Missing Persons/Exemplar Group.

3. A five-digit sample ID number is generated for each donor. The five-digit ID number meets the following conditions:
   i. It falls within the numerical range 00000 to 99999, inclusive
   ii. It is generated randomly each time a new swab is collected.
   iii. It is unique to all other assigned ID numbers, past or present.

4. This number is placed on a large coin envelope that is also labeled with the donor's name. The information is recorded in Lab Types. This number becomes the sample identifier.

5. Lab Types samples are classified as reference materials.

B. Sample Processing

1. Lab Types samples can be processed along with casework exemplar samples.

2. After cutting, the swabs are returned to their envelopes. In most cases, these envelopes are placed in the appropriate container for long-term storage. For situations where samples are not to be stored by Forensic Biology, see the *Sample Disposition* section.

3. Extraction, quantitation, amplification, and STR analysis are performed identically to casework exemplar samples. The results are sent to the Lab Types Custodian.

C. Sample Disposition

1. Lab Types samples and extracts are stored like all other exemplar swabs. In certain circumstances, a swab and extract may need to be destroyed or returned to an individual.

   i. NYPD swabs and extracts will be returned to the NYPD Integrity Control Officer.

2. To return a sample, the envelope is cut open so that the Eppendorf tube containing the sample extract can be inserted along with the swabs. The five-digit ID number written on the envelope is obscured or removed.

3. In circumstances where samples need to be destroyed, the swabs and extract can be disposed of appropriately.

D. Database Maintenance

1. The Lab Types Custodian is in charge of keeping the main Lab Types Databas up to date with all relevant information as results arrive.

2. The information is maintained as an Access database and must include, but is not limited to:

   i. ID number
   ii. department/agency/employer of donor
   iii. Date of swab receipt
   iv. Date and time of extraction, quantitation, and amplification
   v. quantitation value
   vi. STR run name
   vii. DNA profile

E. Lab Types Reference Databases

1. Due to the nature of the information kept in the main Lab Types Databank, the full version is not suitable for general usage by analysts for comparison to evidence profiles. For this reason, copies of the main Lab Types Databank are created with various data fields deleted or hidden from view.

2. Two versions of the main Lab Types Databank are periodically created for routine use by analysts or managers.
   i. One version contains only the ID numbers and the corresponding DNA profiles and is designed for use by analysts for comparison with casework DNA profiles.
   ii. A second version is designed for use by management, and has ID numbers, DNA profiles, and names of sample donors.

3. Each version is spot-checked and write-protected prior to placement online.
   i. To spot-check a truncated version of the database, an authorized analyst other than the Lab Types Custodian checks the database entries against electropherograms of the samples.
   ii. After this has been completed, the copies are created and write-protected. These copies are then directed to the Lab Types Manager for approval and placement on the network for general usage.

F. Searching the Lab Types Database

1. The Lab Types databank in Access can be sorted by genotype at each locus.

2. Double click *LabTypes:Table* on the left side of the screen if the table does not automatically populate.

3. There are two ways to search: manual and filtered. In both tables, profiles are automatically sorted in numerical order from top to bottom across all columns.

4. **Manual Search.** To search manually, an analyst scrolls down until they find the genotype at the locus in the first column.

5. **Filtered Search.** To perform a filtered search, click on arrow next to the locus name at the top of the column. Check each allele or allele combination you wish to search

   This process can be done with as many subsequent loci as necessary. To reset the filter and display the entire database again, click *Toggle Filter* on the Home toolbar

Revision History:

February 9, 2010 – Initial version of procedure.
April 30, 2012 – Revised the "Guiding Principles and Scope" section. Lab Types contains both locally- and nationally-recognized exogenous DNA profiles and are kept under a "contaminant" listing.
July 16, 2012 – Specific terminology was removed and replaced with generic terminology to accommodate LIMS.
October 1, 2012 – A Target Date of 60 days for Lab Types samples was added to "Section A – Sample Collection."
August 14, 2015 – Updated section to reflect current practices.

Preventive Action

GUIDING PRINCIPLES AND SCOPE

Preventive action is a pro-active process to identify opportunities for improvement and potential sources of non-conformities rather than a re-active process to the identification of problems or complaints. Aside from the review of the operational procedures, preventive action may involve analysis of data including trend and risk analyses and proficiency test results.

This document describes the Department's procedure to identify potential preventive actions, either technical or concerning the Management System, and the steps to be taken to deal with the issues identified.

PROCEDURE

1. Any staff member that becomes aware of potential sources of non-conformities in laboratory operations informs their immediate supervisor and/or Assistant Director as soon as practicable.
   a. Immediate supervisors notify their Assistant Director if the AD was not part of the initial notification. The initial process to communicate potential preventive actions up the chain-of-command ensures that any follow-up action is implemented sooner, rather than later.

2. The immediate supervisor and/or Assistant Director investigates the potential problem and conducts a preliminary review of the root cause(s) of any potential non-conformity to determine if action is necessary. The appropriate Technical Leader (if the potential problem is a technical problem), the Quality Assurance Manager, and/or other supervisors/managers may be consulted for assistance.
   a. If the investigating supervisor/manager does not agree that a potential problem exists, no further action is necessary.

3. If the investigating supervisor/manager agrees that a potential problem exists, and if a root cause of the potential non-conformity is determined, the immediate supervisor and/or Assistant Director develops a plan of action to deal with the issue. This may include a change in technical procedures and/or the initiation of new guiding principles. The plan of action shall include the initiation of controls to ensure that the preventive actions are effective. A description of the potential problem, root cause, and plan of action is documented on a **Preventive Action Form** and submitted to the Quality Assurance

Manager. If the preventive action is of a technical nature, the Quality Assurance Manager will forward the form to the appropriate Technical Leader for review.

4. If the preventive action is of a technical nature, the appropriate Technical Leader either approves the plan or decides on an alternate arrangement.

   If the preventive action concerns a potential non-conformity in the Management System, the Director or his/her designee either approves the plan or decides on an alternate arrangement.

5. The Preventive Action Form and any associated documentation (such as Manual Change Forms, copies of emails, etc.) are filed with the Quality Assurance Unit.

6. The Quality Assurance Manager reviews the Preventive Action Form within six months to determine if the preventive action plan that was put into place has been effective.
   a. The Quality Assurance Manager records their evaluation of effectiveness on the Preventive Action form, e.g., a notation that none of the anticipated non-conformities had occurred.
   b. If the action plan is determined to have been effective, the preventive action is considered to be complete.
   c. If the action plan is determined not to have been effective, the Quality Manager will determine whether the changes made as a result of the action plan need to be discontinued or revised.

Revision History:
February 9, 2010 – Initial version of procedure.

Proficiency Testing Program

GUIDING PRINCIPLES AND SCOPE

Proficiency tests are given to qualified analysts to evaluate both their individual competence and the quality performance of the laboratory. Proficiency tests must be analyzed using only approved methods and/or procedures. While there are several types of proficiency tests, the Department of Forensic Biology utilizes open-external proficiency testing and blind reanalysis proficiency testing.

The proficiency testing program is designed to meet the requirements of ASCLD/LAB and the Quality Assurance Standards for Forensic DNA Testing Laboratories. The external proficiency testing program is not just a requirement; it is also a quality assurance measure used to monitor performance and identify areas in which improvement may be needed.

External DNA proficiency tests are obtained from New York State and ASCLD/LAB approved proficiency test providers, for example, Collaborative Testing Service (CTS), Orchid Cellmark (IQAS), and the College of American Pathologists (CAP).

Serology results are reported on DNA tests obtained from CTS.

PROCEDURE

A. DNA Open-External Proficiecy Testing Program

1. All analysts, technical reviewers, and technicians undergo semiannual external proficiency testing to the full extent in which they perform each technology in casework. Technology refers to the type of forensic DNA analysis performed (i.e. STR, Y-STR, mtDNA.) The program is administered in an open proficiency-testing format and in accordance with the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories.

2. One test is assigned to each participant in the first six months of the calendar year and the second test is assigned in the last six months of the calendar year.
   a. The interval between consecutive tests must be at least four months and cannot exceed eight months.
   b. **The laboratory uses the assigned date/start date to calculate the interval between tests**.

c. Newly qualified individuals enter the external proficiency testing program within six months of the date of their qualification.

3. The scheduling of external proficiency tests is completed by a member of the Quality Assurance Unit prior to the start of each calendar year. While minor changes may be made during the year (test vendor, paired analyst, addition/removal of personnel, etc.), the schedule of each analyst/technician is not changed unless a change is necessary due to an extended leave of absence.

4. All specimens of an external proficiency test are analyzed according to current standard operating procedures. However, some exceptions are made in order to comply with the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories. For example, the following sample types, which during normal casework analysis might only be tested in one or two multiplex reactions, must be amplified at all CODIS core loci or CODIS core sequences and tested in all applicable technologies (Autosomal STR, Y-STR, and/or Mitochondrial DNA) to the full extent that the analyst participates in casework:

   1) Excluded suspects
   2) Mixtures, even if there are other clean profiles
   3) Epithelial cell fractions from an unknown stain or from a body orifice swab, even if the results match the victim type.

5. The laboratory utilizes a team approach for casework testing. Therefore, proficiency tests are conducted in the same manner. However, each individual is proficiency tested at least once per year in each methodology to the full extent of his or her participation in casework.

   Methodology refers to analytical procedures used to support a DNA-typing technology [i.e. extraction methods (manual v. automated,) quantification methods, typing test kits and instrument platforms]. The extent in which each individual participates in casework may be team dependent.

   Individuals who perform STR, YSTR, and/or mtDNA amplification, analysis and/or review must perform these skills twice per year per technology.

   a. Unlike other titles, Criminalist Level I's are competent only in selected areas of the analytical process and their competency differs between the different teams within the laboratory. Criminalist I's cannot interpret the final DNA typing data or prepare an associated written scientific report.

Thus, their participation in proficiency tests is limited to the methodologies that they are competent in and they are paired with a DNA Analyst on proficiency tests.

b. Individuals using both manual and automated methods are proficiency-tested in each at least once per year.

6. A laboratory report to summarize the results of the Proficiency Test is written by the DNA interpreting analyst. The DNA interpreting analyst is also responsible for completing any vendor paperwork to document the results. The DNA interpreting analyst must ensure that the data transcribed to the vendor's paperwork is accurate. The proficiency test file is then forwarded to the analyst's supervisor, manager, and/or designee for a full technical review.

7. In addition to conducting a full technical review of the proficiency test file, the reviewer(s) must also review the completed vendor's paperwork to ensure that data has been transcribed correctly.

8. After the proficiency test has been completed (including a full administrative review), the DNA interpreting analyst assigned to the proficiency test is responsible for delivering the test results to the test vendor. The delivery method may vary from vendor to vendor, but is typically either by fax or e-mail.

9. After official results have been received by the proficiency test provider, a designated Quality Assurance Unit member grades the tests.

a. Non-administrative discrepancies on proficiency tests that affect typing results and/or conclusions should be reported to the appropriate Technical Leader at the time of discovery. If confirmed, the Technical Leader must inform the CODIS Custodian/Supervisor so that appropriate follow-up action can be initiated. A formal QUALITY INCIDENT REVIEW may be required.

10. All proficiency-test participants are informed of their final test results. Participants are required to sign the appropriate area on the Proficiency Test Evaluation Form to document that they have received and have been informed of the final test results.

11. After the grading of all proficiency tests within the series, the designee informs the appropriate Technical Leader of the results of all participants.

B. Serology Open-External Proficiency Testing Program

Serology is a sub-discipline of the Biology discipline (as per ASCLD/LAB). The laboratory will endeavor to arrange for each employee to annually complete a serology proficiency test, but it is not required to do so.

Forensic Biology proficiency tests purchased from CTS allows the participant to report results for serology tests as well as for DNA testing. Therefore, serology proficiency testing is satisfied in this manner. The management of this test is identical to the management of DNA external proficiency tests – tests are reported, reviewed, and participants are evaluated in the same manner.

C. Blind Re-analysis Proficiency Testing Program

1. The Blind Re-analysis Proficiency Testing Program is a quality assurance program where a previously examined sample is re-examined by a different analyst to check for correctness of the initial examination and results.

2. **DNA Blind Reanalysis Program.** The Quality Assurance Unit is responsible for reanalyzing DNA samples, reviewing the results, and comparing them to the original analyses.
   a. Each month, a minimum of two (2) exemplar samples are selected from cases completed within the previous year.

b. Each sample is submitted for extraction, quantitation, amplification (in at least one casework multiplex system), analyzed for STR results, and the results compared to the original results. Re-examined results are documented separate from the case file and maintained as a record by the Quality Assurance Unit.

c. A second reanalysis must be performed if the results are not concordant. All follow-up actions must be documented and maintained.

3. **Serology Blind Reanalysis Program.** The laboratory has a blind serology re-analysis program for negative cases. The purpose of this program is to ensure that negative serology results are accurate.

a. Approximately 25% of negative sexual assault kits are selected by a Quality Assurance member for re-analysis. The re-analysis must occur prior to the release of any report.

b. Re-analysis of negative serology cases is conducted by casework analysts that are not involved in the original analysis. Each sample within the case that was previously analyzed is re-analyzed to ensure consistent results and checked for correct itemization. Examination notes and confirmatory-test results are compared. Original and re-examined results are retained in the case file.

c. If discrepancies between results occur, the Quality Assurance Manager and/or the Quality Assurance Supervisors must be contacted to determine what follow-up action is necessary. All follow-up actions must be documented and maintained.

Revision History:

February 9, 2010 – Initial version of procedure.

March 30, 2012 – Removed the requirement to use the Proficiency Test Review Form to document the review of proficiency tests (consistent with current practice).

July 16, 2012 – Specific forms and worksheets were removed and replaced with generic terminology to accommodate LIMS.

August 14, 2015 – Updated percentage of cases chosen for Blind Reanalysis per year, as well as changing “QA supervisor” to “designated QA member”.

Quality Incident Review

GUIDING PRINCIPLES AND SCOPE

Action must be taken when major departures from the policies and procedures in the Management System have been identified. These quality incidents shall be identified and reported so that appropriate follow-up action can be implemented. The identification of problem areas and subsequent preventive actions will improve the quality of our Department.

This document describes the Department's process for dealing with quality incidents when major departures from the policies and procedures in the Management System have been identified. Problems or difficulties can arise in all phases of laboratory operations, and these must be evaluated and dealt with appropriately. Listing each potential problem is impractical, and this topic is considered in general terms.

Technical errors or problems related to casework testing are dealt with as per the CONTROL OF NON-CONFORMING TESTING procedure. The procedure provides direction with respect to when such problems must be dealt with via a Root-Cause Analysis.

This procedure ensures that, when required, our accrediting bodies and/or appropriate entities are notified in a timely manner.

PROCEDURE

A problem with the Management System of the laboratory may be identified through a variety of activities such as internal or external audits, management reviews, feedback from customers, and from staff observations.

Not every quality incident or departure from Management System policies and procedures is serious enough to require a Quality Incident Review.

It is impossible to anticipate all situations in which a Quality Incident Review must be conducted; therefore, sound judgment is required in determining the extent and level of reporting and documentation required.

Issues which require a Quality Incident Review include, but are not limited to:

- Systemic non-conformance with the policies and procedures in the Management System (e.g., failure to properly document staff qualifications and training)

The Quality Assurance Manager should be consulted if there is any question as to whether a Quality Incident Review is required.

A. Quality Incident Reporting

1. All staff members are responsible for reporting apparent quality incidents that come to their attention.
   i. All quality incidents are reported to the staff member's immediate supervisor.
   ii. Supervisors or managers who become aware of any non-technical quality incidents, either directly or through notification from other staff members, must proceed to Step 2.
   iii. Any member of staff who believes that the potential quality incident is of major concern but is concerned about confidentiality, may inform the Quality Assurance Manager immediately and directly

2. The supervisor or manager investigates the issue to determine the details of the potential problem.

3. If the initial investigation indicates that a quality incident occurred, but that a formal Quality Incident Review is not needed, the investigating supervisor/manager shall inform the Quality Assurance Manager of the incident via email.

4. If the initial investigation indicates that a formal Quality Incident Review is needed, the investigating supervisor/manager consults with the Quality Assurance Manager.

5. The Quality Assurance Manager determines whether to proceed with a formal Quality Incident Review.

6. The Quality Assurance Manager informs the investigating supervisor/manager of the decision.

7. The decision not to proceed with a formal Quality Incident Review does not prevent a supervisor or manager from conducting other follow-up action, e.g., counseling of an individual.

B. Quality Incident Review

1. The Quality Assurance Manager assigns a supervisor or manager to conduct the Quality Incident Review.

2. The Quality Incident Review (QIR) form guides the steps of the process.

3. The incident is described in detail on the QIR, including the effect(s) of the discrepancy.

4. The assigned supervisor/manager conducts an investigation to determine the **root cause** of the incident. The root cause(s) may not be obvious and thus a careful analysis of all potential causes of the problem is required. Potential causes could include, but are not limited to, problems with:

   - customer requirements
   - the samples
   - sample specifications
   - methods and procedures
   - staff skills and training
   - consumables, or
   - equipment and its calibration.

   The assigned supervisor/manager conducting the QIR shall use the OCME Root Cause Analysis Procedure as a guide to determine the root cause of the incident.

5. Follow-up actions are proposed to correct the immediate problem and minimize the potential for recurrence of the problem. Corrective actions may include, for example, personnel counseling, modifying procedures or forms, etc. The follow-up action plans should also include:

   - The parties responsible for implementing the corrective actions
   - The monitoring that will be conducted to ensure that the proposed actions have been effective. Very serious and/or systemic issues may require follow-up audits of the affected areas of activity.

6. The QIR is forwarded to the Quality Assurance Manager for review and approval of the proposed corrective actions.

C. Close-out of Quality Incident Reviews

1. The completion of corrective actions is documented on the QIR.

2. If the monitoring activities indicate that the initial follow-up actions were insufficient to address the quality incident, the Quality Assurance Manager may initiate additional follow-up actions.

3. The Quality Assurance Manager determines which incidents must be disclosed to accrediting bodies and/or appropriate entities.

4. The QIR is "closed" when monitoring activities are completed and all individuals agree that corrective action(s) have been satisfactorily implemented and effectively addressed the quality issue.

5. The QIR and any supporting records are filed with the Quality Assurance Unit.

Revision History:

February 9, 2010 – Initial version of procedure.

September 24, 2010 – Added step in procedure to document quality incidences that do not rise to the level of a Quality Incident Review on the Non-conformity Reporting Form.

April 1, 2014- Any references to technical non-conformities have been removed from the QIR and will now solely be addressed via the Non-Conforming Work Procedure.

Reagents

GUIDING PRINCIPLES AND SCOPE

A reagent is any substance used because of its chemical or biological activity. Reagents are used directly, or at a dilution, in a given analytical procedure. Reagents are different than *chemicals*, which are used in the preparation of in-house reagents.

Only reagents suitable for the methods employed may be used in the Department of Forensic Biology. This procedure describes in general terms the requirements for the documentation and quality control of commercial reagents and for the formulation, documentation, and quality control of in-house reagents. The last section in this document is a list of the reagents used by the Department.

PROCEDURE

Reagents are classified into two general categories:

A **critical reagent** is determined by empirical studies or routine practice to require testing on established samples before use on evidentiary or casework reference samples in order to prevent unnecessary or irreparable loss of sample. "Critical reagents" includes a variety of test kits or systems used in DNA testing.

A **non-critical reagent** is a reagent whose failure to work properly will not cause irreparable loss of sample. Therefore, the use of a QC test procedure to check the reliability of the reagent prior to its use in casework is not an absolute requirement, but will be performed by the Department on a reagent-by-reagent basis.

Reagents are prepared **in-house** or are obtained **commercially.**

Personnel preparing reagents, and those who use reagents, are to exercise care at all times to ensure that no exogenous DNA will be introduced to a stock reagent.

A. Reagents Prepared In-House

1) Reagents are prepared in-house according to an approved formula or procedure. Reagent preparation is usually performed by a member of the Quality Assurance Unit.

2) A **reagent sheet** form exists for every reagent prepared in the laboratory and is used as a guide for the preparation of the reagent.

3) Each reagent record contains the following information:
   i. the identity of the reagent
   ii. date of preparation
   iii. identity of individual preparing the reagent
   iv. standard batch size
   v. ingredients of the reagent
   vi. data entry section

4. Some reagent records (such as critical reagents) may also include:
   i. lot numbers
   ii. expiration dates (see step 6)
   iii. quality control procedures (aka, "reliability checks") to be performed and passed before the reagent is released for use in the laboratory.

5. Reagents prepared in the laboratory are labeled with, at a minimum:
   i. the identity of the reagent
   ii. the lot number
   iii. the expiration date (see step 6)

   When a reagent is aliquotted into tubes that are too small to be labeled with all of the required information, each tube is marked with the identity of the reagent and its lot number and stored in a "cryobox" that is labeled with the required identifying information listed above.

6. The expiration date given is usually one year from date of make/aliquot or the earliest expiration date of the reagents being used, whichever comes first. This may also be stated in each reagent forms.

7. Staff is notified via email by the Quality Assurance Unit regarding reagents that are expiring.

B. Commercial Reagents

2. Commercial reagents include, but are not limited to, kits for DNA quantitation and genetic typing.

3. A **Raw Materials** form exists for each commercial reagent that requires quality testing prior to use in casework. The applicable quality control procedure is contained on the form.

4. Commercial reagents are labeled with, at a minimum:
   i. The identity of the reagent
   ii. The expiration date as provided by the manufacturer or as determined by the laboratory.
      1) If identical reagents with the same lot number are assigned different expiration dates by the manufacturer, then the expiration date will be extended to the latest date provided that it passes quality control testing.

         For example, Lot #1234 of a regent was received on June 1, 2011 (Bottle A) and has a manufacturer assigned expiration date of June 1, 2012. A second bottle of Lot #1234 was received on December 1, 2011 (Bottle B) and has has a manufacturer-assigned expiration date of December 1, 2012. Since the manufacturer supports the use of this particular lot of reagents until December 1, 2012, the expiration date of Bottle A will be extended to December 1, 2012 provided that Bottle B passes quality control testing.

      2) Commercial reagents without an expiration date provided by the manufacturer shall expire two years *after receipt* unless otherwise indicated**.**

C. Reagent Quality Control Testing

Quality control (QC) tests are reliability checks and may be used by the Department to ensure that reagents are performing as expected. If needed, these tests must be completed prior to the reagent being used in actual casework. A reliability check may be a combination of several quality control tests and, for ease of classification, are assigned QC testing procedure numbers. If a reagent sheet lists a "procedure" for its quality control test, then the reagent must pass all the quality control tests listed below. If it only lists a specific "QC" number, then the reagent must pass that quality control test only.

	QC Tests Included	Analysis
Procedure 1	QC615, QC616 or QC620	Real Time Quantitative PCR
Procedure 2	QC240, QC350	PCR Amplification and STRs
Procedure 3	QC145A, QC615/620, QC350	Organic Extraction, Real Time Quantitative PCR, PCR Amplification, and STRs
Procedure 4	QC145/165, QC160, QC615/620, QC350	Chelex/M48 Extraction, Real Time Quantitative PCR, PCR Amplification, and STRs
Procedure 5	QC350	3130xl STRs

D. Reagent Records

Reagent records, such as reagent sheets and Raw Materials Forms are a form of Quality Record, and shall be stored in accordance to the guiding principles and procedures that govern such records. See CONTROL OF RECORDS in the Administrative Manual for further information.

E. REAGENTS USED BY THE DEPARTMENT

This section shows a list of reagents used in the Department of Forensic Biology. The list includes reagents prepared in-house as well as commercial reagents. Each reagent is classified as "Critical" or "Non-Critical".

REAGENT	CRITICAL
a-Amylase powder from Human Saliva	N
Acid Phosphatase Test Reagent	Y
Alkaline Substrate Buffer	Y
Agilent DNA 1000 Kits	N
AmpFℓSTR Identifiler PCR Amplification Kit	Y
AmpFℓSTR MiniFiler PCR Amplification Kit	Y
AmpliTaq Gold DNA Polymerase Kit (all components)	Y
BigDye Terminator Cycle Sequencing Kit	Y
BSA Solution, 5 mg/mL	Y
Centrisep columns, strips, and plates	N
Chelex, 20%	Y
Chelex, 5%	Y
Deoxynucleotide Triphosphates, 2.5 mM (dNTPs)	Y
Digest Buffer	Y
Dithiothreitol (DTT), 1M	Y
DMSO	N
EB1	Y
EB2	Y
EDTA, 0.5 M	N
EDTA, 0.5M for WTC	Y
ExoSAP-IT	Y

REAGENT	CRITICAL
Fish Sperm DNA	Y
Genetic Analyzer Buffer (ABI)	N
HiDi Formamide	N
Human Leukemia 60 (HL60)	Y
Hydrogen Peroxide, 3%	N
Kastle-Meyer (KM) Reagent	Y
MagAttract DNA Mini M48 Kit (Qiagen)	Y
Magnesium Chloride (MgCl2)	N
Nuclear Fast Red	Y
Organic Extraction Buffer	Y
PBS for Chelex Extraction	Y
PBS for Nail Extraction, 25mM EDTA	Y
PBS Solution for Seratec (PBS tablets)	Y
PBS Solution, Irradiated (LCN DNA)	Y
Phase lock gel tubes	N
Phenol Chloroform Isoamyl Alcohol (PCIA)	Y
Picric Indigo Carmine (PIC)	Y
POP-4	N
POP-6	N
Poly A RNA	Y
Primer, FBI – A1, B1, C1, D1, C2, D2, A4, B4, HVIF, HVIR, HVIIF, HVIIR (mtDNA)	Y
Proteinase K solution	Y
Quantifiler Trio DNA Quantification Kit	Y
Roche Primer and Reaction Mix	Y

REAGENT	CRITICAL
Saline (0.85% NaCl)	N
SDS, 2%	N
SDS, 20%	Y
SDS, 0.01%, 0.05%, and 1% (LCN DNA)	Y
Seratec PSA Semiquant Kits	Y
Seratec Amylase Forensic Test	Y
Sequencing Loading Buffer	Y
Sodium Acetate, 0.1 M	N
Standard DNA for Real Time Quantitative PCR	Y
Sterile Deionized Water	Y
SYBR Green I	Y
Terg-a-zyme	N
TAE, 1X	Y
TBE buffer	N
Tris-EDTA, 1X	Y
Tris-HCl, 1M (pH 8.0)	N
UltraPure Water	Y
Xylene	N
Yfiler™ PCR Amplification Kit	Y

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Revision History:

February 9, 2010 – Initial version of procedure.
October 28, 2010 – Added the Mag Attract DNA Mini M48 Kit and the MiniFiler PCR Amplification Kit to the list of reagents.
December 29, 2011 – Revised Section B.3 to clarify how the laboratory determines the expiration dates of commercial reagents.
July 16, 2012 – Portions revised to generalize terminology to accommodate LIMS.
August 20, 2012 – Revised Section B.3 to clarify how the laboratory determines the expiration dates of commercial reagents.
April 1, 2014 – Revised Section A to include clarification of expiration dates of Reagents made in-house. Replaced YM1 STR with Yfiler™ PCR in Critical Reagent list.
November 24, 2014 – Updated wording for reagent Expiration dates. Added EDTS, 0.5M for WTC to the critical reagent list and replaced Irradiated Water with UltraPure Water.
February 2, 2015- Updated Section E. Added Quantifiler and Seratec Reagents, Removed outdated reagents from the Reagent List.
May 1, 2015 – Updated QC procedures to reflect use of QC615 (QC with Quantiflier Trio).
August 14, 2015 – Updated procedure 1 with new QC616 protocol and corrected reference to location of the Control of Records procedure.

Validation

GUIDING PRINCIPLES AND SCOPE

Validation is the process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis. It is the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected. Only validated methods and procedures may be used with casework samples.

This is different from a performance check, which is a quality assurance measure to assess the functionality of laboratory instruments, equipment, and software that affect the accuracy and/or validity of forensic sample analysis.

The validation process identifies the critical aspects of a procedure which must be carefully controlled and monitored. Validation studies must have been conducted by the Department of Forensic Biology prior to the adoption of a procedure by our laboratory. This procedure describes the requirements of the validation process.

PROCEDURE

All staff members are encouraged to propose new technologies, methodologies, or procedures to be used in casework. Proposals may be forwarded to the Forensic Biology Future Technologies Planning Team. The Director shall make a final determination on whether or not to validate any proposed new technology, methodology, or procedure.

Validations are a planned activity, and the exact tests of one validation may differ from another depending on the new technology, methodology, or procedure being tested. The appropriate Technical Leader shall be consulted to determine which studies must be conducted to ensure efficacy and reliability for forensic casework use. If the technology, methodology, or procedure concerns DNA testing, the Technical Leader must ensure that the appropriate tests, as listed in the FBI's Quality Assurance Standards for Forensic DNA Testing, are conducted.

Validation plans may differ from the initial assessment of the Technical Leader. They may be updated as development proceeds.

While not required, prior to starting any validation, a preliminary assessment may be done to ensure the time and effort that will be dedicated to the validation will be worthwhile.

A. Developmental Validation

1. Developmental validation is the acquisition of test data and determination of conditions and limitations of a ***new or novel*** methodology for use on forensic samples.

2. If another laboratory's developmental validation studies are being used, appropriate documentation or citations for these studies must be available.

3. Developmental validation studies must include the following, where applicable:

   i. Testing using case-type samples, including samples from adjudicated cases or mock samples that mimic casework samples
   ii. Characterization of genetic marker
   iii. Sensitivity, stability, and species specificity studies
   iv. Reproducibility studies
   v. Population studies, such as allele frequency distributions and independence of the population databases
   vi. Mixture studies
   vii. Precision and accuracy studies
   viii. PCR-based studies, including reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies.

4. All developmental validations conducted by the Department must include an executive summary, which summarizes all the studies conducted. The executive summary must include specific recommendations (such as settings, quality assurance parameters, interpretation guidelines, or mixture interpretation guidelines) and must include a statement as to whether the method is fit for the intended use. While not required, it is recommended that each study conducted have an individual summary of results.

B. Internal Validation

1. Internal validation is an accumulation of test data within the laboratory to demonstrate that ***established*** methods and procedures (such as forensic DNA methods or procedures that are published in peer reviewed articles) perform as expected in the laboratory.

2. Prior to implementing a new or revised methodology or procedure, the Department must first demonstrate the reliability of the method or procedure internally. This includes changes in detection platform, changes in DNA test kits, or the implementation of new body-fluid identification procedures. Internal validation studies must be sufficient to support and document the reliability of the method or procedure and must include the following, where applicable:

   i. Testing using known samples
   ii. Testing using non-probative evidence samples or mock evidence samples
   iii. Reproducibility and precision
   iv. Sensitivity and stochastic studies
   v. Mixture studies
   vi. Contamination assessment

3. As a result of the internal validation studies, quality assurance parameters, interpretation guidelines, and mixture interpretation guidelines (where applicable) shall be defined.

4. The documentation of an internal validation includes an executive summary, which summarizes all the testing conducted. The executive summary must include specific recommendations (such as settings, quality assurance parameters, interpretation guidelines, or mixture interpretation guidelines) and a statement as to whether or not the method is fit for the intended use. While not required, it is recommended that each study conducted have an individual summary of results.

C. Review and Approval of Validation

1. Completed validation project packages are submitted to the appropriate Technical Leader for review and approval. The package includes:
   i. Test records and all required summaries
   ii. Draft technical procedure

2. All validations must be reviewed and approved by the appropriate Technical Leader before the technology and/or procedure is used in casework.

Note: Approval of a validation does not necessarily denote that a technology or procedure is online for casework. Training needs, budgetary concerns, etc., must be taken into consideration before the technology or procedure is implemented.

3. At the Technical Leader's discretion, the technology or procedure may be used on select cases prior to lab-wide implementation. However, the technology or procedure are not be used on any casework until standard operating procedures are written and have been approved by the appropriate Technical Leader.

D. Training

Training commences after approval of the validation by the appropriate Technical Leader. The initial training of analysts can be considered a "dry-run" of the procedure, and the technology, methodology, and/or procedure are not used in casework until all concerns that may be raised during the initial training have been addressed.

E. Storage of Validation Records

Records of validation studies are stored by the Quality Assurance Unit indefinitely. In general, validations that have been reviewed by an external audit team will be stored on the fourth floor of the DNA Building (Records Storage), while validations that have not been reviewed by an external audit team will be stored within the operational areas of the Quality Assurance Unit. However, general convenience and spacing issues may alter the exact location of any validation study.

Revision History:

February 9, 2010 – Initial version of procedure.