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	QC620	Real Time Quantitative PCR
Procedure 2	QC240, QC350	PCR Amplification and STRs
Procedure 3	QC145A, QC620, QC350	Organic Extraction, Real Time Quantitative PCR, PCR Amplification, and STRs
Procedure 4	QC145/165, QC160, QC620, 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 Quality Assurance/Quality Control 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 MagAttract 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 3, 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.

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.