OSBI Forensic Biology Unit Policy Manual

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Forensic Biology Unit Quality Manual

ATTENTION: If any portion(s) of the Forensic Biology Unit Policy Manual is/are unclear to any analyst or if a circumstance arises outside the scope of this document, it is the responsibility of each analyst to immediately notify the Technical Manager and the respective Supervisor to seek clarification/approval or obtain guidance on the issue BEFORE proceeding.

Per requirements in the FBI's Quality Assurance Standards for DNA Databasing Laboratories 11.2 and the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories Standard 11.3, ***"Except as otherwise provided by state or federal law, the laboratory shall have and follow written procedures to ensure the confidentiality of the database, known or casework reference samples and the information in DNA databases and DNA records. Except as otherwise provided by state or federal law: reports, case files, DNA records, and databases shall be confidential."*** **DNA profiles are not to be transmitted outside the OSBI network electronically. The OSBI network (e-mail and server) is secure; therefore, NDIS allows these electronic transmissions. However, DNA profiles will not be e-mailed outside of the OSBI network without prior authorization from the Technical Manager.**

Forensic Biology Unit Quality Manual

FBUQM_1 SCOPE (top ↑)

1.1 The Forensic Biology Unit (FBU) Policy Manual shall apply to work specifically performed by analysts/technicians working within an officially defined Forensic Biology Units or by analysts/technicians working in the capacity as a Forensic Biologist or associated technician.

1.2 Unless specifically stated, the Forensic Biology Unit (FBU) Quality Manual (QM) applies to DNA casework only.

1.3 The purpose of this policy manual is to establish and maintain a documented quality system that is appropriate to the Forensic Biology Units of the Oklahoma State Bureau of Investigation (OSBI). The CODIS Unit maintains a separate quality manual which can be found in the CODIS Policy Manual.

1.4 Any document referenced within the policy manual shall be available to each Forensic Biology laboratory within the OSBI multi-laboratory system (per FBI Quality Assurance Standards (QAS) Audit Document discussion).

1.5 Hyperlink and attachment maintenance (removal, addition, or correction of hyperlinks and attachments) shall not constitute a new document revision.

ATTACHMENT(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

REFERENCE(S):
OSBI CODIS Unit Policy Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_2 DEFINITIONS (top ↑)

Terms in this document follow the definitions set forth in Section 2 of the Quality Assurance Standards (QAS). In addition, the OSBI Forensic Biology Units establish the following definitions:

Amplification Set - may contain samples and reagent blanks from different cases and/or questioned and known samples together

Analytical Threshold - the relative fluorescence units (RFU) value above which an observed well-formed peak that is not the result of a diagnosable issue such as stutter, pull-up, spike, minus A, etc. can be assumed to be observed due to the presence of an allele

Annual – once per calendar year

Concurrent – taking place at the same time with the same conditions [as clarified by the Federal Bureau of Investigation (FBI)]

Differential Extraction – process for extracting DNA from all samples testing presumptive or confirmatory positive for seminal fluid

DNA Analysis - using the Polymerase Chain Reaction (PCR) to examine Short Tandem Repeats (STR) utilizing capillary electrophoresis

Dye blobs - disassociated primer dyes which are reproducible artifacts often observed with a particular lot of amplification kits

Extraction Set - may contain samples from different cases and associated reagent blanks; however, questioned and known samples must be in separate extraction sets

Instrument Baseline Threshold - the RFU value below which a peak is indistinguishable from the baseline noise

Instrument Spike - non-reproducible artifacts which occur occasionally within an electophoretic run resulting in the appearance of anomalous peak(s) on the electropherogram. A peak resulting from an instrument spike is easily identified as such upon close examination. Such a peak will usually be composed of all colors of approximately equal apparent fluorescent intensity and occur at the same data point and/or scan number in GeneMapper ID-X. **Intimate Sample** - originating from the body (i.e. vaginal swabs, swab of hand, oral swabs, etc.)

Limit of Detection (LOD) – as it pertains to genetic analysis, this is the RFU value below which a peak is unable to be brought up to a peak height useful for interpretation through increased injection time(s). This may or may not be equivalent to the baseline threshold; however, it cannot be less than the baseline threshold and should be relatively close to the baseline threshold to minimize any data loss.

Low Copy Number (LCN) DNA Analysis – as referred to at the OSBI, LCN DNA analysis shall be defined as any DNA analysis where additional amplification cycles beyond the OSBI's internal validation, post-amplification purification, reduced reaction volume, nested PCR, or an increased injection time or voltage outside the scope of the OSBI's internal validation were performed; the OSBI does not perform LCN DNA analyses and, as such, any sample subjected to these conditions will not be submitted to NDIS.

Microvariant Alleles - alleles which contain an incomplete repeat unit (i.e. 9.3 at TH01)

Minus A (-A) – a reproducible artifact of the PCR process generated by the inability of AmpliTaq Gold™ to catalyze the addition of a single nucleotide (predominantly adenosine) to the 3' end of the PCR product during the final extension step

Mixture – two or more contributors are present in a DNA profile

Off-ladder Allele (OL) - an allele whose size is not represented within the allelic ladder

Off-scale Peaks (OS) - those reproducible peaks above the linear detection of the instrument; any fluorescence signal that exceeds the detection threshold of the instrument. The OS quality flag indicates if the camera is saturated at any data point within the analysis range. It is not based on an absolute RFU value (peak height).

Peak Height Ratio (PHR) - the ratio of the peak with the lowest RFU to that of the peak with the highest RFU of a heterozygous pair expressed as a percent (truncated)

Peak Stochastic Threshold (PST) – the RFU value above which it can be assumed that peak "drop-out" does not occur and any observed allelic peak that has no sister allele present can reasonably be believed to be homozygous

Process Step – this refers to each step in a process of analysis. This would include, but is not limited to, adding protocol specific reagents at listed volumes, use of Microcons, or use of any other equipment in the analysis process,

and, as such, an associated blank must also be taken through the process concurrent to any samples. However, in some process steps such as the concentrating of a sample, although a questioned sample and its associated reagent blank(s) must both be placed into the device, it is acceptable to remove any associated blank(s) from the process step while allowing the sample to continue in the step until an appropriate volume is achieved. Adding reagent to bring any volumes to a working volume is not considered a process step.

Pull-up (PU) - when the matrix cannot compensate for an excess of one color, causing the "pulling up" of peaks in another color; in other words, the instrument is not able to resolve spectral overlap. This may be observed as a small peak at a similar scan number as a large peak in another color.

Quantitation Negative Control (QNC) - is used to detect DNA contamination of the quantitation reagents. This control consists of only quantitation reagents without the addition of template DNA.

Reagent - any substance or mixture used in the analysis process of the Forensic Biology Units. Chemicals used to make reagents are not considered "reagents", but rather chemicals (i.e. NaOH). Reagents will have an expiration date of one year from the date of receipt, unless otherwise specified by the manufacturer or in the FBU Policy Manual and/or its attachments.

Review Date - the completion date of the review of the protocols

Serology - the identification of body fluids including blood, semen, and other biological materials

Single Source - one contributor is present in a DNA profile

Standard Extraction - all samples except those requiring a differential extraction

Stutter (ST) - a reproducible artifact of the PCR process; a minor product peak longer or shorter than the corresponding main allele peak. The stutter peak may be various sizes in relation to the main allele peak dependent on the repeat structure and the amplification kit.

Technical Manager – equivalent to "Technical Leader" as defined by QAS

Trailing Primer Peak – peaks associated with the primer peak that fall within the size calling range

Trainee – an analyst or technician undergoing training in a particular discipline (i.e. serology or DNA analysis)

Trisomy – three alleles at a single source STR locus

True Allelic Peak (allele) - a relatively symmetric pointed arch in an upward direction. It is wider at the base than at the summit and shows an area of non-linear taper to and from the highest point. Generally, the peak should have a height:width ratio of >1. True allelic peaks should be present in only a single color unless pull-up has occurred.

Virtual Allele - an allele that is labeled by the software, even though it is not present in the allelic ladder. For example, if using the Identifiler® Plus panel in GMID-X, there is an allelic bin for an 11 at D3S1358, although the ladder alleles range from 12 to 19. Thus, an allele labeled as an 11 at D3S1358 would be considered a virtual allele. The term "virtual allele" does not in any way indicate that the allele is not a true allele, but simply indicates it is an off-ladder allele that has been assigned a label.

Work product - the material utilized in testing and/or generated as a function of the analysis process

Working Volume – the volume of a sample extract or a control that is the minimum volume required for appropriate DNA analyses to be carried out on the associated extract or batch of extracts. Any addition of liquid to an extract or control for the purpose of bringing that sample up to the working volume does not constitute a dilution. Addition of liquid to any sample or control that is above the working volume constitutes a dilution, and all controls should be handled as required by the FBI Quality Assurance Standards.

Forensic Biology Unit Quality Manual

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_3 QUALITY ASSURANCE PROGRAM (top ↑)

3.1 The OSBI Criminalistics Services Division (CSD) maintains a general, unit-neutral Quality Manual. The OSBI Forensic Biology Units maintain this unit-specific quality manual to specifically address the quality system for forensic serology and DNA testing.

3.1.1 The Quality System will address the following areas:

3.1.1.1 Goals and Objectives

3.1.1.2 Organization and Management

3.1.1.3 Personnel

3.1.1.4 Facilities

3.1.1.5 Evidence Control

3.1.1.6 Validation

3.1.1.7 Analytical Procedures

3.1.1.8 Equipment Calibration and Maintenance

3.1.1.9 Reports

3.1.1.10 Review

3.1.1.11 Proficiency Testing

3.1.1.12 Corrective Action

3.1.1.13 Audits

3.1.1.14 Safety

3.1.1.15 Outsourcing

3.2 **Retention of Records**

The Forensic Biology Units shall follow OSBI CSD Quality Procedure (QP) 16.1 – Control of Records for quality and technical records to include the following areas:

- Proficiency Tests
- Corrective Action
- Audits
- Training Records
- Continuing Education
- Case Files
- Court Testimony Monitoring

According to the OSBI CSD Quality Procedures, the above listed areas are all quality records except case

files which are considered technical records.

3.3 **Annual Review** - The Forensic Biology Units shall conduct an annual review to be inclusive of the overall quality assurance program, analytical procedures, quality control procedures, training manual, and any other division (CSD) or agency (OSBI) procedures that are applicable to the quality of DNA analysis.

3.3.1 FBU Level Documents: The Technical Manager shall direct and document his/her approval of the annual review of the Forensic Biology Unit Quality Assurance Program. This review may be an ongoing process of incorporating any deviations or suggestions submitted through OSBI CSD QP 3 – Deviations and QP 15 – Preventive Action into the next revision of the FBU Policy Manual. A final review of the incorporated changes will be completed before issuance, and approval documented in the Approval section of the document. The Technical Manager will conduct the annual review by requesting assistance through the Biology Subcommittee, as needed.

3.3.1.1 The final, documented approval of the Annual Review shall correspond sequentially or identically in date with either the issuance of a new policy revision, approved by the Technical Manager, to the current policy or by the insertion of the "Review Date" and the phrase "Annual Review" within the History section of the current policy. Changes to the policy shall be outlined in the History section.

3.3.2 CSD/OSBI level documents: Review of these policies/procedures will be documented in a memo, and any proposed deviations/changes will be submitted to the appropriate authority.

3.4 **Goals and Objectives** - The OSBI Forensic Biology Units shall strive to accomplish the following goals and objectives:

3.4.1 Provide the customers of OSBI laboratory services access to forensic biology testing in accordance with the services provided as described in the OSBI CSD Quality Manual.

3.4.2 Ensure the quality, accuracy, and validity of all forensic biology testing, examination documentation, reporting, and courtroom presentations.

3.4.3 Ensure the integrity of evidence through evidence handling, evidence processing, and chain of custody documentation.

3.4.4 Maintain national accreditation and NDIS (National DNA Index System) participation.

REFERENCE(S):
OSBI CSD Quality Manual

FBUQM_4 ORGANIZATION AND MANAGEMENT (top ↑)

4.1 The OSBI Forensic Biology Units managerial staff shall:

4.1.1 Have the authority and resources needed to meet the requirements of the Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS).

4.1.1.1 In the event that the managerial staff does not have the authority or resources to ensure

that the standards are being met, the Technical Manager shall suspend DNA laboratory operations, utilizing OSBI CSD QP 13 – Non-Conforming Work, until such time that the DNA testing at one laboratory or multiple laboratories re-establishes the managerial authority or resources necessary to meet or exceed these standards.

4.1.2 The Technical Manager of the OSBI Forensic Biology Discipline is responsible for all Technical aspects of the OSBI's multi-laboratory Forensic Biology system. The Forensic Biology system is comprised of two separate laboratories: Forensic Science Center — FSC Forensic Biology (FBU - major crimes), FSC Specialized Forensic Biology (SFBU - property crimes and cold cases), and CODIS Units and the Northeast Regional Laboratory — NERL Forensic Biology.

4.1.3 There is a single casework CODIS Administrator responsible for the entire OSBI multi-laboratory system.

4.1.4 Each laboratory is a distinct facility with at least two full time DNA analysts.

4.1.4.1 In the event that a laboratory ceases to have two full time DNA analysts, the Technical Manager shall immediately suspend the processing of any new DNA casework at the specific facility. The sole qualified DNA analyst shall complete all open DNA cases. The specific facility shall continue to process serology cases and perform required quality procedures until a second full time DNA analyst is qualified.

4.1.5 OSBI Policy 103 – Authority and Responsibility and OSBI CSD QP 1 – Responsibilities and Authority specify the responsibility and authority of each position within the OSBI Forensic Biology Unit chain of command. In addition, the Forensic Biology Unit QM 5 section titled "Personnel" reflects the responsibilities associated with each position.

4.1.5.1 The Organization and Management Structure of the OSBI Criminalistics Services Division (CSD) is detailed in the OSBI CSD Quality Manual. The Forensic Biology Discipline "Organization and Management Structure" chart (QM_OrgChart) maintained by the OSBI Forensic Biology Units reflects the interrelation of all appropriate personnel.

4.1.6 The document titled "Technical Manager Contingency Plan" reflects the plan to maintain uninterrupted Technical leadership over the DNA testing operations within the OSBI Forensic Biology system. Exclusion from the document shall not exclude qualified individuals from participating in a contingency plan during a Technical Manager position vacancy.

ATTACHMENT(S):
FBU Policy Manual QM_OrgChart
OSBI Memo titled "Forensic Biology Discipline Technical Manager Contingency Plan"

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBUQM_5 PERSONNEL (top ↑)

5.1 The OSBI Forensic Biology Units shall be staffed with personnel employed by the agency that meet the qualifications contained within this section as well as within any documents referenced herein and the qualifications listed in the QAS.

5.1.1 Analyst duties and responsibilities are outlined in a general written job description for each of the Criminalist (I, II, III, IV, and V) positions including responsibilities, duties, and skills necessary for the position. The general written job description is maintained by the Office of Personnel Management of the State of Oklahoma. Additional Selective Qualifications for analysts within the OSBI Forensic Biology Units are on file with the OSBI Human Resources Division. Criminalist IVs in regional facilities may or may not also serve as DNA analysts.

5.1.1.1 Any analysts who are hired to perform forensic DNA analysis that do not meet the qualifications listed in the general job description for a Criminalist position, the Selective Qualifications for the Forensic Biology Discipline, or the QAS will be brought into compliance with these requirements prior to commencing any independent forensic DNA analysis. For required higher education classes, a passing grade will be a "C" or higher. QAS-required classes previously completed with a grade lower than "C" will have to be retaken. Failure to successfully complete with a grade of "C" or higher may result in progressive discipline up to and including termination.

5.1.2 All analysts and technicians shall complete a documented training program for all procedures that the analyst or technician will perform. The training program will comply with OSBI CSD QP 19 – Training and meet the requirements set forth by the QAS. The training program may be modified based on an analyst's/technician's prior experience and with Technical Manager approval. A passing grade for all exams and quizzes in the training program will be 80%, unless deemed otherwise and documented by the Technical Manager.

5.1.2.1 Trainees will be required to successfully complete all applicable sections of the OSBI Forensic Biology Unit Training Manual, including mock case(s) utilizing a range of samples routinely encountered in casework, mock court, and technical question assessments before release for independent casework. The Memo releasing the analyst or technician for casework will include the scope of the release including testing while the "Authorization to Work" Memo will include testing, instrumentation, equipment, testimony, etc. as required by ASCLD/LAB.

5.1.2.2 The mock case(s), mock court and technical question assessments are considered "competency test(s)". The successful completion of the competency assessment will be documented by the Technical Manager in the trainee's training materials. If an analyst or technician has previous forensic experience, the previous experience will be reviewed by the Technical Manager for adequacy and applicability to OSBI FBU procedures.

Adequate previous training may result in documented modifications to the FBU Training Program for the trainee.

5.1.3 All analysts qualified to perform forensic DNA analysis will be required to attend continuing education training as outlined in the QAS.

5.1.3.1 All training attended, whether conducted internally, externally, through multi-media, or internet delivery, to meet continuing education requirements must meet the standards listed in the QAS and attendance must be documented. The laboratory will maintain records associated with any such training, as required by the QAS.

5.1.3.2 The literature review program approved by the Technical Manager per OSBI CSD QP 19 – Training shall consist of quarterly literature reviews over topics relevant to forensic biology. Literature review documentation will be maintained by the Technical Manager.

5.1.3.3 The OSBI laboratory will maintain physical or electronic access to a collection of current books, reviewed journals, and/or other literature applicable to DNA analysis.

5.1.4 Records for qualifications, training, skills, and experience shall be retained in accordance with OSBI CSD QP 16.1 – Control of Records. The Technical Manager shall have access to documentation of qualifications, training, skills and experience that is pertinent for the analysis of DNA and should have access to the same documentation as is pertinent for serology.

5.2 Technical Manager

5.2.1 Any individual filling the position of OSBI Forensic Biology Technical Manager must meet the minimum education, experience, training requirements, and qualifications as per the QAS; in addition, all requirements of the Criminalist IV classification maintained by the Office of Personnel Management of the State of Oklahoma shall also be met. The Technical Manager position shall be filled by a full time employee of the OSBI.

5.2.2 The OSBI Forensic Biology Technical Manager shall have at least three years of forensic DNA laboratory experience obtained at a laboratory where forensic DNA testing was conducted for the identification and evaluation of biological evidence. Any Technical Manager appointed or hired on or after July 1, 2009, shall have a minimum of three years of human-DNA experience as a qualified analyst on forensic samples. The OSBI Forensic Biology Technical Manager shall complete (within one year of appointment) the FBI-sponsored auditor training.

5.2.3 The duties, responsibilities and authority of the OSBI Forensic Biology Technical Manager will, at a minimum, be those designated by the QAS. Additional duties, responsibilities and authorities can be required of the Technical Manager as necessary to meet the needs of the agency.

5.2.4 The Technical Manager will be available to provide on-site, telephone or electronic consultation as needed. The Technical Manager shall conduct a site visit semi-annually to all satellite labs performing DNA analysis in a laboratory system.

5.2.5 A newly appointed Technical Manager will be responsible for the documented review of the following: analyst qualifications (training records and education), validation studies, and methodologies currently used in the laboratory within the first year.

5.3 Casework CODIS Administrator

5.3.1 Any individual filling the position of OSBI Casework CODIS Administrator must meet the minimum educational, experience, and CODIS training requirements listed in the QAS.

5.3.2 The OSBI Casework CODIS Administrator shall be a currently or previously qualified casework DNA analyst with documented mixture interpretation training or, if appointed or hired prior to July 1, 2009, the Casework CODIS Administrator shall have documented mixture-interpretation training and documented completion of FBI-sponsored auditor training.

5.3.3 The OSBI Casework CODIS Administrator shall complete the FBI auditor training within one year of appointment, if not previously attended, and shall participate in the FBI-sponsored CODIS software training within six months of appointment, if not previously attended.

5.3.4 The duties and responsibilities of the OSBI Casework CODIS Administrator will, at a minimum, be those designated by the QAS. Additional duties and responsibilities can be required of the Casework CODIS Administrator as necessary to meet the needs of the agency.

5.3.5 The Casework CODIS Administrator has the authority to terminate an analyst's and/or laboratory's participation in CODIS if there is an issue identified with the reliability or security of the CODIS data.

5.3.6 For the OSBI, the Casework CODIS Administrator position will be filled by the CODIS State Administrator. In the event that the Casework CODIS Administrator position is unoccupied, no casework profiles will be uploaded to NDIS.

5.4 DNA Analyst

5.4.1 All analysts performing DNA analysis must meet the minimum educational and experience requirements listed in the QAS.

5.4.1.1 Any analyst meeting the minimum educational requirement of the QAS with a class titled other than those listed will have the documented approval of the Technical Manager for the substitution. Compliance with the standard may be demonstrated with review of any pertinent materials by the Technical Manager such as transcripts, syllabus, a letter from the instructor, or other document(s) that support(s) the course content.

5.4.2 Each analyst will have at least 6 months of documented, forensic human-DNA laboratory experience before release for independent forensic DNA casework.

5.4.3 Analysts shall successfully complete a competency test before beginning independent analysis.

5.5 Technical Reviewers

5.5.1 Each Technical Reviewer shall be a current or previously qualified analyst in the methodologies being reviewed.

5.5.2 Each Technical Reviewer shall successfully complete a competency test prior to participating in the technical review of data. This competency test is incorporated as an assignment into the Forensic Biology Training Program.

5.5.3 Each Technical Reviewer shall participate in the external proficiency testing on the same

technology, platform, and typing amplification kit used to generate the DNA data being reviewed.

5.6 Laboratory Technicians

5.6.1 Laboratory technicians shall have the equivalent of an Associate's Degree with some laboratory experience.

5.6.2 Laboratory technicians shall have documented training specific to their job function(s).

5.6.3 Laboratory technicians shall successfully complete a competency test before participating in DNA analysis on evidence.

5.7 Laboratory Support Personnel

5.7.1 Any laboratory support personnel shall have documented training specific to their job function(s).

ATTACHMENT(S):
OSBI Memo titled: "Additional Selective Qualifications for Biologist Position with the OSBI"

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories
FBU Selective Qualifications Memo

FBUQM_6 FACILITIES (top ↑)

6.1 Each laboratory within OSBI's multi-laboratory Forensic Biology system will follow OSBI CSD QP 20 – Lab Security to insure the integrity of analyses and evidence.

6.2 Pre-amplification activities such as evidence examination, DNA extraction, and amplification set-up will be separated by time and/or physical space from each other.

6.3 Each of the forensic biology laboratories maintain a separate room or rooms for the generation, processing, and storage of amplified DNA product separate from evidence examination, DNA extraction, and amplification set-up areas. The door between the amplification room and other areas will remain closed except for passage between areas.

6.4 The procedures for cleaning and decontaminating facilities and equipment are specifically addressed in QC_1.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBUQM_7 EVIDENCE CONTROL (top ↑)

7.1 The OSBI Forensic Biology Discipline follows OSBI CSD QP 5 – Evidence Intake, QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance and QP 7 – Evidence Transactions to ensure the integrity of physical evidence.

7.1.1 Evidence (defined by the OSBI CSD Quality Manual) shall be clearly distinguishable from all other evidence at all times. When batching is used, case numbers and item numbers shall be present on all work products in addition to the original packaging and evidence in accordance with the OSBI CSD Quality Procedures.

7.1.1.1 Work products (defined in FBU QM_2) shall be handled according to OSBI CSD QP 6.1 – Evidence Handling and the appropriate sections below.

7.1.1.2 Serology Work Products—Utilized in Testing

Cuttings, microcentrifuge tubes, manufactured assay cards, slides prepared for blood confirmatory testing, and substrate materials for serological tests (Takayama, AP Spot Test, Sperm Cell Search, Seratec p30 card) are considered work products utilized in testing. Following the completion and documentation of the test results, these work products shall be discarded.

7.1.1.3 Serology Work Products—Generated as a Function of the Analysis Process

Slides prepared for sperm cell searches are considered work products generated as a function of the analysis process. Following the completion and documentation of the test results, these work products shall be handled according to the retention policies within the OSBI Forensic Biology Units.

7.1.1.4 DNA Work Products—Utilized in Testing

Swabs, cuttings, and portions of biological samples are considered work products utilized in testing. Following the completion and documentation of the test results, these work products shall be handled according to the retention policies within the OSBI Forensic Biology Units.

7.1.1.5 DNA Work Products—Generated as a Function of the Analysis Process

- Extracts, dilutions, normalized dilutions, and amplified DNA products (tubes, plates) containing amplified DNA and genetic analyzer plates containing amplified DNA are considered work products generated as a function of the analysis process.
- Following the completion and documentation of the analysis method, extracts, dilutions, and normalized dilutions shall be handled according to the retention policies within the OSBI Forensic Biology Units.
- Following the completion and documentation of the technical review, amplified DNA products (tubes, plates) and genetic analyzer plates containing amplified DNA shall be discarded.

7.1.2 The chain of custody will be kept in accordance with OSBI CSD QP 5 – Evidence Intake, QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance and QP 7 – Evidence Transactions.

7.1.3 Evidence and work product in progress will be handled in accordance with OSBI CSD QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance in order to minimize loss, contamination, and/or deleterious change.

7.1.4 Secured, controlled access areas for evidence storage and work product in progress are outlined in OSBI CSD QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance.

7.2 Retaining or returning evidence or work products shall be handled according to the retention policies outlined in OSBI CSD QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator, and Freezer Maintenance and QP 16.2 – Contents of Case Records and as guided by Oklahoma Statutes (Title 22 §1372).

7.3 Disposition and Consumption of Evidence

7.3.1 The OSBI Forensic Biology Units shall report the disposition of evidence for all evidence submitted to the Forensic Biology Units for analysis.

7.3.2 The OSBI Forensic Biology Units shall follow OSBI CSD QP 6.1 – Evidence Handling and QP 16.2 – Contents of Case Records as well as the requirements in CASE_1.1.12 and CASE_2 for the handling of samples requiring consumption for analysis.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBUQM_8 VALIDATION (top ↑)

8.1 The OSBI Forensic Biology Units shall use only validated methodologies for DNA analyses. The OSBI Forensic Biology Units shall define within the validation plan, in addition to the requirements of QP 21.2 – Validations, whether the proposed validation is developmental or internal.

8.2 If the validation is developmental, the OSBI Forensic Biology Units shall follow QAS Standard 8.2.

8.2.1 If the validation is for serology only, the OSBI Forensic Biology Units shall follow OSBI CSD QP 21.2 – Validations.

8.3 Internal Validations

If the validation is internal, the OSBI Forensic Biology Units shall follow QAS Standard 8.3. For internal validations, the OSBI Forensic Biology Units may obtain the developmental validation documentation from the original laboratory or company or establish through citation and publications that the original developmental validation sufficiently addressed Standard 8.2.1 prior to beginning the internal validation.

8.3.1 In accordance with the QAS and the OSBI CSD QP 21.2 – Validations, the OSBI Forensic Biology Units shall complete internal validations utilizing the following general order of events: (1) submit validation plan per OSBI CSD QP 21.2 – Validations, (2) perform system and site-specific validation studies, (3) submit documentation and summary for approval, (4) complete individual competencies, and (5) release validated procedure for use in forensic DNA casework.

8.3.2 Internal validation of all methods shall be reviewed and approved by the Technical Manager prior to implementing a new procedure.

8.3.3 Internal validation studies shall include the following, as applicable:

- known and non-probative evidence samples or mock evidence samples
- reproducibility and precision studies
- sensitivity and stochastic studies
- mixture studies
- contamination assessment
- NIST traceable samples

8.3.4 Internal validation studies within the OSBI Forensic Biology multi-laboratory system shall be divided into two categories: system and site-specific. All validations including summaries and data shall be maintained electronically on a secure network server accessible by all forensic biology laboratories. The laboratory responsible for the system internal validation study shall incorporate their site-specific internal validation study into the system study. The site-specific

validation studies shall include at least precision, sensitivity, and contamination assessment studies.

8.3.5 Quality assurance parameters and interpretation guidelines shall be defined in the internal validation and included within the specific analytical procedure.

8.4 Individual Competency – All analysts shall successfully complete a competency test, assigned by the Technical Manager, to the extent of his/her/their participation in casework analyses before beginning independent analysis utilizing a new methodology. Depending on the methodology being introduced, the competency test may consist of written, oral, and/or practical testing.

8.5 Introduction of Modified Procedure – Modifications which would require a protocol change shall be evaluated by comparison with current procedures using similar samples. These modifications must be documented and reviewed by the Technical Manager. Approval of the modification for use will occur through the issuance of a deviation in accordance with OSBI CSD QP 3 – Deviations or through the issuance of a new protocol revision in accordance with OSBI CSD QP 2 – Document Control.

8.6 Critical Instruments

8.6.1 Introduction of Critical Instruments - Purchase of an additional critical instrument (of the same model as another critical instrument previously validated) requires a performance check. Purchase of a new critical instrument (of a different model or with a change in the analysis software, etc.) requires internal validation of the new instrument.

8.6.2 Modification to Critical Instruments - The Technical Manager should evaluate each critical instrument modification to determine the level of evaluation (performance check versus internal validation) required prior to using the modified critical instrument in casework. Modifications that do not affect the analytical process by the instrument require only a performance check.

8.7 Modifications to Instrument Software - Software modifications that do not impact interpretation or the analytical process require a performance check prior to implementation. New software or software modifications that impact interpretation, the analytical process, or sizing algorithms require internal validation prior to implementation. The Technical Manager should evaluate each software modification to determine the level of evaluation (performance check versus internal validation) required prior to using the modified software in casework.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBUQM_9 ANALYTICAL PROCEDURES (top ↑)

9.1 This document and corresponding documents represent the Forensic Biology Units' analytical procedures. This document will be reviewed annually by the Technical Manager. Documentation of changes will be noted in the history section, and previous revisions will be archived.

9.1.1 The Forensic Biology Units shall follow the standard operating procedures outlined in this document and the corresponding documents. OSBI Forensic Biology DNA analytical procedures shall contain the following, as applicable:

- Type of Test
- Reagents and Supplies
- Sample Preparation
- Extraction Method
- Equipment (Critical Equipment as listed in FBU QM_10 section 10.4.1)
- Controls
- Individual Steps of the Procedure
- Interpretation of Results

9.2 Reagents

9.2.1 The OSBI Forensic Biology Units shall follow the OSBI CSD QP 8 – Ordering, Receiving, and Verifying Reagents and Supplies.

9.2.2 In addition to the labeling requirement of OSBI CSD QP 8 – Ordering, Receiving, and Verifying Reagents and Supplies, the name of the reagent must be on the container.

9.2.2.1 If changes have been made to the product insert, the Technical Manager shall be notified.

9.2.2.2 Items that are too small to legibly record all the required information are exempt from the labeling and date opened requirements, as long as the storage container is labeled with all the appropriate information. Generally, this only applies to tubes from kits.

9.2.2.3 Reagents shall be considered expired at 11:59 p.m. on the expiration date. Reagents with a manufacturer's expiration date of month/year shall expire at 11:59 pm on the last day of the month.

9.2.2.4 All supporting documentation for function verification tests should include the reagent name or set of reagents (if tested together), date, initials, and page number.

9.2.3 The OSBI FBU will document the following on the appropriate QCR Chemical Formulation Worksheets for each in-house reagent:

- Chemical Formulation and Preparation (Amounts of chemicals for each reagent are provided as the standard formulation. If the final volume of the reagent that is made is to be different than that listed, the amounts of each ingredient are to be changed such that the proportions of the chemicals remain unaltered).

- Storage
- Function Verification
- Unsatisfactory Reagent Performance
- Expiration (If any ingredient of the reagent has an earlier expiration date than the reagent expiration, the expiration date shall be determined by the ingredient expiration date.)

9.2.3.1 The OSBI FBU will document the following on the appropriate QCR Chemical Receipt/Function Verification Worksheets for commercial reagents:

- Receipt Information
- Storage
- Function Verification
- Unsatisfactory Reagent Performance
- Expiration (Expiration date or guidance as provided by the manufacturer or as determined by the laboratory. If the expiration date is determined to be longer by the OSBI Forensic Biology Units, the justification documentation shall be maintained with the quality records for the reagent.)

9.3 Critical Reagents

9.3.1 Critical Reagents-Serology - No reagents utilized for serological testing shall be considered critical.

9.3.2 Critical Reagents-DNA

- TE^{-4}
- Stain Extraction Buffer
- Proteinase K and DTT
- PrepFiler® Express / PrepFiler® Express BTA Extraction Kit
- Differential Extraction Reagents (Organic and AutoMate)
- DNA Quantitation Kits
- DNA Amplification Kits (including thermostable DNA polymerase, primer sets, and allelic ladders)

9.4 The OSBI Forensic Biology Units shall quantify the amount of human and/or human male DNA in all casework samples prior to STR or Y-STR DNA amplification.

9.5 The criteria for the evaluation of controls and standards shall be specified in the applicable analytical procedures. The OSBI Forensic Biology Units shall, at a minimum, adhere to the following requirements.

9.5.1 Standards shall be used during the quantitation procedure(s).

9.5.2 Positive and Negative Amplification Controls associated with the forensic samples being typed shall be amplified concurrently in the same instrument with the samples and typed at all loci using the same primers as the forensic samples.

9.5.3 Reagent Blank Control

9.5.3.1 After a thorough evaluation of your extraction batch, determine (based on your training and experience) the appropriate number of reagent blanks for the set. At least one reagent blank control must be included with every set of extractions. All reagent blanks should be placed at the end of the extraction set.

9.5.3.2 If analysts are splitting an extraction set into mini-sets, the reagent blank for each mini-set should be placed after that mini- set and remain designated to those samples.

9.5.3.3 Reagent blank controls shall be extracted concurrently and in the most sensitive volume of the extraction set.

9.5.3.4 Reagent blanks should be brought to a working volume of ~30uL. If a sample is concentrated, an associated reagent blank must also be concentrated. No sample or reagent blank may be concentrated to a volume less than the maximum volume of the amplification kit to be used.

NOTE: Some reagent blanks may be brought to a working volume of approximately 15uL, depending on related sample types based on analyst's discretion (i.e. a robust sample with no need for future additional DNA analysis).

9.5.3.5 All reagent blanks must be quantitated.

9.5.3.6 If a reagent blank replicate in an extraction set demonstrates any signal as a result of quantitation, the reagent blank with the most signal must be amplified and characterized.

9.5.3.7 If all reagent blanks in an extraction set produce no signal during quantitation, then the reagent blank closest to the samples should be amplified and characterized first.

9.5.3.8 For extraction sets being amplified, an associated reagent blank(s) must be amplified using the same primers as the forensic sample(s), in the same instrument model as the forensic sample(s).

9.5.3.9 In certain instances it may be necessary to split the extraction of a sample across multiple extraction tubes (i.e. if four vaginal swabs have little sperm detected, it may be desirable to extract all four swabs, but this would exceed the space available in any single extraction tube). In such instances, one reagent blank is acceptable for this set of simultaneous extractions. If the analyst combined two extracts from two separate extraction sets (different times/dates), then the reagent blanks from each extraction set must be combined as well.

9.5.3.10 The maximum volume, per amplification kit, of the reagent blank should be amplified.

9.5.3.11 If a sample is re-amplified with the same amplification kit and the template volume does not increase over that of the original reagent blank and does not alter the amplification parameters to increase sensitivity, then the reagent blank associated with the extraction set does not need to be re-amplified.

9.5.3.12 Reagent blanks must be typed using the same instrument model as the forensic samples;

if injected at varying injection times, the reagent blank must satisfy the most sensitive injection conditions. The reagent blank must be injected at 15 seconds, but can also be injected at the most sensitive injection conditions during the first set of injections.

9.5.3.13 Any DNA extract produced prior to July 1, 2009, may be amplified even if there is insufficient or no remaining associated reagent blank, as long as the analysis data associated with the reagent blank(s) has/have been evaluated and is/are included in the case file. Cases in this category should involve Technical Manager consultation.

9.5.4 **Controls**

9.5.4.1 **Quantitation Negative Control**

9.5.4.1.1 This control must be included with each plate of samples prepared for quantitation at the same time.

9.5.4.1.1.1 The QNC should be "undet.". If a value is detected, the Technical Manager will be consulted to determine if the plate is acceptable or if it will be re-setup and re-run.

9.5.4.2 **Negative Amplification Control**

9.5.4.2.1 This control must be included with every amplification set.

9.5.4.2.2 The negative amplification control used in the OSBI Forensic Biology Units shall substitute TE^{-4} in place of sample.

9.5.4.2.3 The negative control should result in no observable peaks above the peak amplitude threshold. If peaks are detected, then reference section FBU QM_9.7 below.

9.5.4.3 **Positive Amplification Control**

9.5.4.3.1 This control must be included with every amplification set.

9.5.4.3.2 The OSBI Forensic Biology Units shall use control DNA provided in the amplification kits (9947A for Identifiler® Plus and 007 for Yfiler®).

9.5.4.3.3 The positive control should yield the expected profile (9947A for Identifiler® Plus or 007 for Yfiler®). If it does not yield the correct profile, the sample set should be re-setup and the Technical Manager will be notified.

9.5.5 The Forensic Biology Units will check their DNA procedures annually or whenever substantial changes to a procedure are made by running NIST/NIST traceable standard reference material. The NIST standard must be, at a minimum, amplified and characterized with each technology in use in the lab (STR, Y-STR, etc.). NOTE: This check must be performed for each instrument type per laboratory annually, but not necessarily on every instrument in the laboratory (i.e. not necessary on all Genetic Analyzers in each laboratory, but must be performed on a 3500 in each laboratory).

9.6 For interpretation of data, verification of control results, and mixture interpretation see CASE_5, and for statistical interpretation see CASE_6.

9.7 Detection and Control of Contamination

The OSBI FBU shall monitor contamination, follow decontamination protocols to minimize/prevent contamination, and follow procedures for interpreting data potentially affected by contamination. In addition, the OSBI FBU shall follow procedures for uniformly evaluating controls and samples to verify expected results as well as remediating instances of control failure or sample contamination. Interpretation of data potentially affected by contamination or failed PCR controls will be evaluated on a case-by-case basis, in conjunction with the supervisor and the Technical Manager. The data provided by the OSBI internal validation of interpretation thresholds will be taken into account during these types of evaluations. Based on an OSBI FBU internal validation, no peaks below the following thresholds need to be evaluated as being attributable to contamination:

- Blue Dye 15 second injection : 70rfu (ID+) 180rfu (Yfiler)
- Green Dye 15 second injection: 100rfu (ID+) 160rfu (Yfiler)
- Yellow Dye 15 second injection: 110rfu (ID+) 130rfu (Yfiler)
- Red Dye 15 second injection: 140rfu (ID+) 140rfu (Yfiler)

ATTACHMENT(S):
FBU Policy Manual QCR_DNA_AMPKIT
FBU Policy Manual QCR_DNA_Automate_Ecell_Digest_Buffer
FBU Policy Manual QCR_DNA_Automate_EXTReagents
FBU Policy Manual QCR_DNA_Formamide
FBU Policy Manual QCR_DNA_GABuffer
FBU Policy Manual QCR_DNA_OrgEXTReagents
FBU Policy Manual QCR_DNA_OrgSAReagents
FBU Policy Manual QCR_DNA_PCI
FBU Policy Manual QCR_DNA_PrepFiler_Kits
FBU Policy Manual QCR_DNA_QUANTKIT
FBU Policy Manual QCR_DNA_QUANTSTNDS
FBU Policy Manual QCR_DNA_SAReagents
FBU Policy Manual QCR_DNA_TE-4
FBU Policy Manual QCR_SER_APSpot
FBU Policy Manual QCR_SER_Hem
FBU Policy Manual QCR_SER_p30
FBU Policy Manual QCR_SER_RSID
FBU Policy Manual QCR_SER_TAK
FBU Policy Manual QCR_SER_Xmas

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_10 EQUIPMENT CALIBRATION AND MAINTENANCE (top ↑)

10.1 The OSBI Forensic Biology Units shall use equipment suitable for the methods employed.

10.1.1 In the event that the OSBI Forensic Biology Units do not have suitable equipment for the

methods used at the OSBI, the Technical Manager shall suspend DNA laboratory operations, utilizing OSBI CSD QP 13 – Non-Conforming Work, until such time that the DNA testing at one laboratory or multiple laboratories re-establishes the suitability of the equipment.

10.1.2 The OSBI Forensic Biology Units shall follow OSBI CSD QP 24 – Calibration and Handling of Equipment.

10.2 Documentation

10.2.1 Any service, maintenance, calibration, or repair performed on laboratory equipment must be recorded on the equipment and maintenance log and maintained by the laboratory.

10.2.2 A performance check/calibration must be documented.

10.2.3 Laboratory equipment shall be used as recommended by the manufacturer or as specified in analytical protocols.

10.3 Unsatisfactory Equipment or Instrument Performance

- If laboratory equipment does not function as expected, OSBI CSD QP 24 – Calibration and Handling of Equipment will be followed.
- The Technical Manager, supervisor, or designee shall make arrangements for needed service or replacement of the unit.
- Service performed on a unit shall be recorded on the equipment maintenance log and maintained by the laboratory.

10.4 Critical Equipment

10.4.1 The following will be considered critical instruments/equipment and shall require annual performance checks as per the quality control procedures:

- Thermometer traceable to national or international standard(s) that is used for conducting performance checks
- Balance/scale
- Robotic Systems
- Thermal Cycler temperature verification system
- Thermal Cycler including quantitative-PCR
- Genetic Analyzers
- Mechanical pipettes

10.4.2 Robotic systems, genetic analyzers or thermal cyclers (including quantitative-PCR) that have undergone repair, service or calibration, and new critical instruments/equipment shall undergo a performance check before use in casework analysis, and this performance check shall be performed according to the appropriate quality control procedure.

10.4.3 A preventative maintenance visit will not be considered "service" and will not require a performance check afterwards, unless the laser is replaced or aligned, or the optics are adjusted.

10.5 Schedule and Maintenance Program

10.5.1 The schedule and maintenance program for instruments and equipment shall follow the requirements in the quality control procedures for equipment.

10.5.2 The records associated with the instrument or equipment should be portable for permanent instrument or equipment transfers within the multi-laboratory system.

10.5.3 In the event an instrument or equipment is permanently removed from service, the records should be archived and accessible for historical purposes.

ATTACHMENT(S):
FBU Policy Manual QC_EquipMain
FBU Policy Manual QC_3500Maint_Log
FBU Policy Manual QC_7500
FBU Policy Manual QC_9700_0.2
FBU Policy Manual QC_9700_0.5
FBU Policy Manual QC_Automate_Maint_Log
FBU Policy Manual QC_Cent
FBU Policy Manual QC_DecontamLog
FBU Policy Manual QC_H2O
FBU Policy Manual QC_pHMeter
FBU Policy Manual QC_PipetCal
FBU Policy Manual QC_PipetCalReprod
FBU Policy Manual QC_Temp
FBU Policy Manual QC_Therm

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_11 REPORTS (top ↑)

11.1 The OSBI Forensic Biology Units shall follow OSBI CSD QP 16.2 – Contents of Case Records and OSBI CSD QP 28 – Report Writing to maintain case record documentation and issue reports.

11.1.1 The OSBI Forensic Biology Unit "Abbreviation List" shall be maintained according to OSBI CSD QM.

11.2 Required Report Elements

11.2.1 Serology Casework Reports shall include, at a minimum, the elements required by OSBI CSD QP 28 – Report Writing.

11.2.2 DNA Casework Reports shall include, at a minimum, the elements required by OSBI CSD QP 28 – Report Writing

11.2.3 Only one Forensic Biologist should accept the responsibility for the content of a report. This shall be documented by the use of a secure electronic signature through the use of the LIMS following OSBI CSD QP 28 – Report Writing.

11.3 The OSBI FBU shall follow OSBI CSD QP 16.1 – Control of Records for case files and OSBI CSD QP 33 – Release of Case Information for report dissemination and distribution of case related information.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_12 REVIEW (top ↑)

12.1 All Serology reports and DNA reports shall meet the administrative and technical review guidelines as established in OSBI CSD QP 31 – Reviews.

12.1.1 In addition, all Serology and DNA reports shall be technically reviewed by a qualified analyst other than the examining analyst.

12.1.2 Technical reviewers shall be or have been a qualified analyst in the methodology being reviewed and shall be released for technical reviews at the discretion of the Technical Manager.

12.1.3 If the technical reviewer is a previously qualified (not currently qualified) analyst, they must participate in a proficiency test as a technical reviewer.

12.2 Completion of the casework technical review shall be documented and the technical review of forensic casework shall include reviewing the following elements, at a minimum:

12.2.1 All case notes, all worksheets, and the electronic or printed data supporting the conclusions

12.2.2 All DNA types to verify that they are supported by the raw or analyzed data

12.2.3 All profiles to verify correct inclusions and exclusions (if applicable) as well as a review of any inconclusive result for compliance with laboratory guidelines

12.2.4 All controls, internal lane standards and allelic ladders to verify that the expected results were obtained

12.2.5 Statistical analysis, if applicable

12.2.6 The final report's content to verify that the results/conclusions are supported by the data. The report shall address each tested item.

12.2.7 For the CODIS technical review portion, verification must be present that all profiles entered into CODIS are eligible and have the correct DNA types and specimen categories. This information should be documented on separate CODIS Data Entry Form as listed below.

12.2.7.1 Prior to the samples being uploaded or searched in SDIS, verification of the eligibility for CODIS should be completed and the correct DNA types and appropriate specimen category should be documented on the CODIS Data Entry Form.

12.2.7.2 For entry into a searchable category of SDIS, the eligibility for CODIS, correct DNA types, and the appropriate specimen category must be verified by two concordant

assessments by a qualified analyst and technical reviewer. This information should be documented on the CODIS Data Entry Form, if applicable.

12.2.7.3 In the OSBI Forensic Biology Units, this verification of eligibility for CODIS, correct DNA types, and the appropriate specimen category is accomplished by the casework analyst filling in all applicable areas of a CODIS Data Entry Form and signing the form electronically. Once signed, the CODIS Data Entry Form is technically reviewed along with the other materials in the case record. During the technical review, the technical reviewer signs the CODIS Data Entry Form indicating that they have verified the profile and specimen category on the form along with any Match Rarity Statistical Threshold calculations required. After the technical review is complete, the case is routed back to the casework analyst in the BEAST. The casework analyst then forwards the CODIS Data Entry Form to the CODIS Unit. The CODIS Unit will review and upload the profile and print the Specimen Detail Report. The CODIS Unit will sign the CODIS Data Entry Form verifying the profile has been entered. The CODIS Unit will then forward the signed CODIS Data Entry Form and the Specimen Detail Report back to the casework analyst. The casework analyst will sign the CODIS Data Entry Form confirming the profile and specimen category were entered correctly. The completed CODIS Data Entry form shall be uploaded to the BEAST Documents Tab and the Specimen Detail Report may be shredded. At this point the casework analyst can approve the technically reviewed report and close the case.

As a part of verifying eligibility for CODIS, all samples for legal entry should be queried to ensure they are not already in the database using the CoDNA system. If the sample is already in the database, this should be documented in the BEAST narrative.

12.3 Technical and administrative reviews shall be documented as needed in accordance with OSBI CSD QP 31 – Reviews. Per OSBI CSD QP 31 – Reviews, hard copy technical review forms will be obtained by accessing the "Reports" button from the BEAST main screen. Run the "TECH REVIEW: Hardcopy Tech Review Form" report and print the Biology discipline review form. The hardcopy must be placed in the case record (saved to the image vault in the BEAST). This method should only be used for documenting additional technical reviews or if technical difficulties with the BEAST necessitate an alternate method. The review shall include:

12.3.1 A review of the case file and report for clerical errors and for the presence and accuracy of the information.

12.3.2 A review of the chain of custody and disposition of evidence.

12.3.3 A completed Technical Review / Administrative Review form.

12.4 Completion of the Technical Review shall be documented in accordance with OSBI CSD QP 31 – Reviews including: any cases requiring correction will be routed to the appropriate analyst for correction. When

routing cases for correction, the reviewer must include an explanation in the comments field describing what must be corrected. Changes made during or after the review process must be recorded. The changes made to the completed examination records as a result of the review process will be tracked for the Forensic Biology Units using a method sufficient to determine what was changed and who made the change. For the Forensic Biology Units, this may be accomplished by using a corrections folder in the portfolio in the BEAST, a detailed description of the changes made to the case record in the BEAST routing comments field (authored by the case analyst, not the reviewer), and/or lining through with initials (electronically).

12.5 The OSBI Forensic Biology Units shall follow OSBI CSD QP 31 – Reviews for unresolved issues between the analyst and the reviewer.

12.6 The OSBI Forensic Biology Units shall follow the "CODIS Unit Policy Manual" for the verification and resolution of database matches.

12.7 The OSBI Forensic Biology Units shall follow OSBI CSD QP 32 – Testimony to document annual monitoring of courtroom testimony.

12.8 Analysts will route their technical reviews to an assortment of analysts to include all qualified technical reviewers (as available) in a rotation to ensure that all analysts are reviewing one another's casework.

REFERENCE(S):
OSBI CSD Quality Manual
OSBI CODIS Unit Policy Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_13 PROFICIENCY TESTING (top ↑)

13.1 The OSBI Forensic Biology Units shall follow OSBI CSD QP 30 – Proficiency Tests.

13.1.1 Individuals using both manual and automated methods shall be proficiency tested in each, at least once per year, to the full extent in which they participate in casework.

13.1.2 Newly qualified analysts and technicians shall enter the external proficiency testing program within six months of the date of their qualification.

13.1.3 The OSBI Forensic Biology Units shall use the **due date** as the date that the proficiency test was performed. Each analyst and technician shall be assigned and complete his/her own external proficiency test. Laboratories that use a team approach to casework examination may do so, on external proficiency tests. However, all analysts, technicians, and technical reviewers shall be proficiency tested at least twice per year in each of the DNA technologies (STR, Y-STR) and each method (test kits) in which they perform forensic DNA analysis – even if the proficiency test scenario provided by the manufacturer doesn't warrant this type of testing. Per the audit document discussion section, and clarification obtained from the FBI:

All questioned samples as well as any male known samples in a proficiency test will be analyzed using both human and male quantitation kits. Any questioned sample in a proficiency test that has an indication of male DNA present, will be amplified using the Y-STR amplification kit, regardless of the results of autosomal amplification kit(s) or "normal" casework processing procedures.

For serology proficiency tests, each analyst and technician shall complete testing for all serological techniques for which they are currently qualified, regardless of the scenario of the test. Technicians will ask a qualified analyst to interpret their serology test results before they proceed with DNA analysis. This analyst will document their interpretations in the technician's documentation.

Technicians will mark the area in the test portal indicating that no interpretations were made and add a note in the comments section that they are completing the test as a technician and therefore no interpretations are made. Technicians will not be required to complete their proficiency test using the BEAST LIMS system since they are not qualified for casework and will not be generating Criminalistics Examination Reports for the proficiency tests. Instead, technicians will place their completed examination documentation into their individual folders on the QA server.

13.1.4 Analysts and technicians shall type all CODIS core loci for each technology performed during each DNA proficiency test.

13.1.5 Proficiency Tests will be submitted to the manufacturer using the online submission option available.

13.1.6.1 Each analyst will notify the Quality Manager of the completion of their test by sending an e-mail to the Quality Manager with the completed Manufacturer's results forms attached.

13.1.6 Maintenance of Proficiency Test Records will be followed as per OSBI CSD QP 30 – Proficiency Tests.

13.1.7 Evaluation of Proficiency Test Results shall include the following:

- Reported inclusions and exclusions were correct
- All reported genotypes and/or phenotypes are correct according to consensus results or are within the laboratory's interpretation guidelines
- Inconclusive or not interpretable results are consistent with written laboratory guidelines and have been reviewed by the Technical Manager
- All discrepancies/errors and subsequent corrective actions are documented
- All final reports are graded as satisfactory or unsatisfactory
    - A satisfactory grade is attained when there are no analytical errors for the DNA profile typing data
    - Administrative errors and corrective actions are documented

13.1.8 Notification of Proficiency Test Results - Analysts shall be informed of his/her final test results and the notification documented prior to issuance of a subsequent proficiency test.

13.1.9 The Technical Manager shall be informed of the results of all participants and this notification shall be documented as per OSBI CSD QP 30 – Proficiency Tests. The Technical Manager shall inform the casework CODIS administrator of all non-administrative discrepancies that affect the typing results and/or conclusions at the time of discovery.

13.2 External Proficiency Test Provider Stipulation - The laboratory shall use an external proficiency test provider approved by the American Society of Crime Laboratory Directors/ Laboratory Accreditation Board (ASCLD/LAB).

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_14 CORRECTIVE ACTION (top ↑)

14.1 The OSBI Forensic Biology Units shall follow OSBI CSD QP13 – Non-Conforming Work and QP 14.1 – Simple Corrections, QP 14.2 – Corrective Action and QP 14.3 – Non-Conforming Results policies, as well as OSBI Agency Policy #110 – OSBI Progressive Discipline, as needed.

14.2 All corrective actions applicable to casework or proficiency tests shall not be implemented without the documented prior approval of the Technical Manager.

14.3 The Technical Manager shall maintain access to a final version of all corrective actions involving analysts within the Forensic Biology Discipline for corrective actions applicable to casework or proficiency tests.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_15 AUDITS (top ↑)

15.1 The OSBI Forensic Biology laboratory shall be audited annually against the QAS Audit Document. The annual audits must occur every calendar year and be at least 6 months and no more than 18 months apart. All internal audits shall be conducted according to OSBI CSD QP 17 – Audits.

15.2 At least once every two years, an external audit shall be conducted by an audit team comprised of qualified auditors (as per the QAS) from an outside agency.

15.3 For internal audits, at least one auditor or member of the audit team shall be a currently qualified auditor and a currently or previously qualified analyst in the laboratory's current DNA technologies, methods and platform.

15.4 Internal and external DNA Audit documents and, if applicable, corrective action(s) shall be submitted to the Technical Manager for review to ensure that findings, if any, were appropriately addressed.

15.4.1 For NDIS participating laboratories, all external audit documentation and laboratory responses shall be provided to the FBI within 30 days of laboratory receipt of the audit documents or report.

15.5 Internal and external audit documentation shall be retained and available for inspection during subsequent audits.

ATTACHMENT(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories – Audit Document
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories – Audit Document

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_16 SAFETY (top ↑)

16.1 The OSBI Forensic Biology Units shall follow the agency wide OSBI Safety Plan - Policy 121 and all subsections.

16.2 The OSBI's environmental health and safety program shall be reviewed once each calendar year and such review shall be documented in the History Section of the policy.

REFERENCE(S):
OSBI Safety Policy 121
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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FBUQM_17 OUTSOURCING (top ↑)

The Forensic Biology Discipline Technical Manager will be consulted during bid specification composition for input and approval. The bid specifications shall include a statement indicating that the vendor laboratory is responsible for providing analysis as well as testimony on their analyses of the evidence.

17.1 The OSBI Forensic Biology Units will ensure the vendor laboratory complies with the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories and the accreditation requirements of federal law for the outsourcing of DNA related services for cases analyzed with the Cold Case Grant funds.

17.1.1 The following documentation will be maintained from the vendor laboratory:

17.1.1.1 Proof of compliance with the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories and compliance with the accreditation requirements of federal law.

17.2 The Technical Manager of the Forensic Biology Discipline will document and maintain approval of the technical specifications of the outsourcing agreement before it is awarded.

17.3 The OSBI Forensic Biology Units will accept profiles generated by the approved vendor laboratory for upload to CODIS after the Forensic Biology Technical Manager has documented and approved the technical specifications of the outsourcing agreement and/or document the approval of acceptance of the ownership of the DNA data.

17.4 The OSBI Forensic Biology Units will verify the integrity of the data received from a vendor laboratory through the performance of a technical review using the FBU QM_12 procedures and BEAST technical review form (reference FBU QM_12.3).

17.5 Prior to the search of DNA data in SDIS, an analyst, CODIS casework administrator or technical reviewer of the OSBI will review the DNA data to verify specimen eligibility and the correct specimen category for entry into CODIS.

17.6 Prior to the upload of data generated by the vendor laboratory to SDIS or the reporting of search results, an analyst in the Forensic Biology Units qualified in the same technology, platform and typing amplification kit used to generate the data and who participates in the OSBI proficiency testing program in DNA analysis will perform a technical review of the data from the vendor laboratory.

17.6.1 Technical reviews will include, at a minimum, the following elements:

17.6.1.1 A review of all DNA types to verify that they are supported by the raw and/or analyzed data.

17.6.1.2 A review of all associated controls, internal lane standards and allelic ladders to verify that the expected results were obtained.

17.6.1.3 A review of the final report to verify:

17.6.1.3.1 The results/conclusions are supported by the data.

17.6.1.3.2 That each tested item (or its probative fraction) submitted to the vendor laboratory is addressed.

17.6.1.4 Verification of the DNA types, eligibility, and the correct specimen category for entry into CODIS.

17.7 The Forensic Biology Technical Manager, or designee, will perform and document an on-site visit of the vendor laboratory prior to the initiation of analysis. This on-site visit will include an evaluation to ensure the vendor laboratory's ability to perform analysis on outsourced casework. The documentation must include the date the on-site visit was performed, a summary of the visit, and the documentation of the personnel who performed the on-site visit. This Memo will be copied to the CSD Laboratory Director. In accordance with the Quality Assurance Standards for Forensic DNA Testing Laboratories Audit Document discussion, "*in addition to the Technical Manager (or designee) performing an on-site visit, the laboratory may elect to accept information/documentation generated from an on-site visit conducted of the vendor laboratory by an NDIS laboratory using the same technology, platform, and typing amplification test kit as long as it was conducted within the past twelve months.*" If this option is chosen, the Forensic Biology Technical Manager shall have documentation demonstrating the review and approval of the on-site visit, the date the visit was performed, a summary of the visit, and the documentation of the personnel who performed the on-site visit.

If the outsourcing agreement extends beyond one year, an annual on-site visit will be conducted by the Forensic Biology Technical Manager, or designee, as described above.

NOTE: Per e-mail from FBI dated May 11, 2012, clarification was obtained regarding the "*in addition…*" verbiage above – A representative from the FBI stated that we "*may use another NDIS lab[']s on-site for the initial evaluation of the vendor lab. The verbiage "in addition" does not mean you have to do a site visit as well. It was language added to indicate that labs could do their own site visit or accept a visit that another NDIS lab did.*"

17.8 All vendor laboratories must be accredited through the ASCLD/LAB Legacy or ASCLD/LAB International program or an organization that follows the ISO/IEC 17025 guidelines.

17.9 The OSBI Forensic Biology Units shall notify customers in writing prior to shipment of evidence when using an outside vendor laboratory for serology/DNA analyses.

17.10 The OSBI CSD Quality Manager will be provided a copy of the accreditation certificate for any vendor laboratory utilized by the Forensic Biology Discipline.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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NOTE: The Forensic Biology Units will consider the "start date" of analysis as the date the assignment is made to the analyst in the BEAST while the "end date" of analysis will be the date the report of the analysis is approved by the examining analyst in the BEAST.

FBU Serology Standard Operating Procedures

SER_1 HEMASTIX (top ↑)

1.1 Type of Test

Presumptive test for the presence of blood.

1.2 Reagents and Supplies

1) Hemastix (Bayer 2190 or product that is equivalent)
2) Deionized Water

1.3 Controls

1.3.1 The following controls are required to be run and recorded once per lot per analyst on each day that Hemastix is used.

1) A positive control made up of known blood.
2) A negative control consisting of deionized water being added to the reagent pad of the Hemastix.

1.3.2 If a daily control fails, troubleshooting should be performed based on analyst training and experience. Continued failure should be brought to the attention of the unit supervisor and technical manager, and that lot should be removed from use in casework.

1.3.3 Requirement for lot numbers/expirations listed in case record:

1.3.3.1 Hemastix

1.3.3.2 Deionized Water

1.4 Individual Steps of the Procedure

1.4.1 Dry Method

1) Lightly brush the dry reagent pad of the Hemastix on the suspected stain.
2) Add deionized water to the reagent pad.
3) Observe reagent pad within 5 seconds for the presence of a color change.

1.4.2 Wet Method

1) Add deionized water to the reagent pad of the Hemastix.
2) Lightly brush the moistened reagent pad of the Hemastix on the suspected stain.
3) Observe reagent pad and/or stain within 5 seconds for the presence of a color change.

1.5 Interpretation of Results

1) Positive Result: A blue or green color develops on the reagent pad or on the stain.
2) Negative Result: No blue or green color develops within 5 seconds.

ATTACHMENT(S):
FBU Policy Manual QCR_SER_Hem

SER_2 TAKAYAMA (top ↑)

2.1 Type of Test

Confirmatory test for the presence of blood.

2.2 Reagents and Supplies

1) 10% sodium hydroxide
2) Saturated glucose solution
3) Pyridine
4) 0.05M Dithiothreitol
5) Deionized Water
6) Slide and cover slip

2.3 Reagent Preparation

2.3.1 Prepare the Takayama reagent according to either the Option A or Option B combinations.

Takayama Reagent Option A

1) 1 part 10% sodium hydroxide
2) 1 part saturated glucose
3) 1 part pyridine
4) 2 parts deionized water

2.3.2 Takayama Reagent Option B

1) 1 part 10% sodium hydroxide
2) 1 part saturated glucose
3) 1 part pyridine
4) 2 parts 0.05M dithiothreitol

2.4 Equipment

1) Microscope
2) Hot Plate (for option A)

2.5 Controls

2.5.1 The following controls are required to be run and recorded once per lot per analyst on each day that Takayama reagent is used.

1) A positive control made up of known blood.
2) A negative control consisting of Takayama reagent only.

2.5.2 If a daily control fails, troubleshooting should be performed based on analyst training and experience. Continued failure should be brought to the attention of the unit supervisor and technical manager, and that lot should be removed from use in casework.

2.5.3 Requirement for lot numbers/expirations listed in case record:

2.5.3.1 Takayama Reagent – working lot number

2.5.3.2 Known blood used for Positive Control

2.6 Individual Steps of the Procedure

1) Place a small sample from the suspected bloodstain on a microscope slide and place a small cover slip on the sample.
2) Using a pipette, transfer the Takayama reagent (Option A or B) to the microscope slide and allow it to flow under the cover slip until the sample is saturated.
3) If Option A is used, heat the slide gently until there is a change in the color of the material under the cover slip.
4) Observe the slide under the microscope.

2.7 Interpretation of Results

1) Positive result: Formation of pink to red, blade, needle, or rhomboidal shaped crystals.
2) Negative result: No formation of characteristic crystals.

ATTACHMENT(S):
FBU Policy Manual QCR_SER_TAK

SER_3 HUMAN BLOOD TESTING (top ↑)

3.1 Type of Test

Human blood detection test (Note: This test is to be used at analyst's discretion. In addition, this test is to be used in conjunction with Takayama, not as a replacement.)

3.2 Reagents and Supplies

1) Individually wrapped RSID cassettes
2) RSID-Blood Running Buffer
3) RSID-Blood Extraction Buffer

3.3 Control Preparation

The following controls are required to be run and recorded once per lot on each day when performing the test (Note: Once controls are prepared, they can be used until consumed as long as the results are as expected and buffer is not expired):

3.3.1 Positive Control

1) Apply ~ 50 µl of human blood to a sterile cotton swab.
2) Place a cutting from the swab head into a microcentrifuge tube and add 1mL of RSID-Blood Extraction buffer, vortex thoroughly and incubate at room temperature for ~1 hour.
3) Following the extraction, remove the cutting from the tube and place into a tube basket inside the tube or in the perforated tube cap and centrifuge the sample for one minute at ~13,200 RPM, vortex thoroughly.
4) Take ~ 20 µl of the blood extract and place in a fresh microcentrifuge tube and add ~ 80 µl of RSID-Blood running buffer to bring to a total volume of ~100 µl.
5) Load the ~100 µl of the sample into the cassette window.
6) Record results at approximately 10 minutes.

3.3.2 Negative Control

1) Apply ~ 50 µl of de-ionized water to a sterile cotton swab.
2) Place a cutting from the swab head into a microcentrifuge tube and add 1mL of RSID-Blood Extraction buffer, vortex thoroughly and incubate at room temperature for 1 hour.
3) Following the extraction, remove the cutting from the tube and place into a tube basket inside the tube or in the perforated tube cap and centrifuge the sample for one minute at ~13,200 RPM, vortex thoroughly.
4) Take ~ 20 µl of the extract and place in a fresh microcentrifuge tube and add ~ 80 µl of RSID-

Blood running buffer to bring to a total volume of ~100 µl.

5) Load the ~100 µl of the sample into the cassette window.

6) Record results at approximately 10 minutes.

3.3.3 If a daily control fails, troubleshooting should be performed based on analyst training and experience. Continued failure should be brought to the attention of the unit supervisor and technical manager, and that lot should be removed from use in casework.

3.3.4 Requirement for lot numbers/expirations listed in case record:

3.3.4.1 RSID-Blood Cartridge

3.3.4.2 RSID-Blood Extraction Buffer

3.3.4.3 RSID-Blood Running Buffer

3.3.4.4 Known human blood used for Positive Control

3.4 Individual Steps of the Procedure

1) Take a cutting from the swab or material and place it in a microcentrifuge tube.
2) Add ~50 µl of RSID-Blood Extraction buffer, vortex thoroughly, and incubate at room temperature for ~1 hour.
3) Following the extraction, remove the cutting from the tube and place into a tube basket inside the tube or in the perforated tube cap and centrifuge the sample for one minute at ~13,200 RPM, vortex thoroughly.
4) Take ~ 20 µl of the extract and place in a fresh microcentrifuge tube and add ~ 80 µl of RSID-Blood running buffer to bring to a total volume of ~100 µl, vortex thoroughly.
5) Add the ~100 µl of extract to the sample cassette window. Record the results at approximately ten minutes.

3.5 Interpretation of Results-Test Plates

1) Positive result: Formation of lines at both control and test lines at approximately 10 minutes
2) Negative result: Formation of a line at the control region only at approximately 10 minutes

ATTACHMENT(S):
FBU Policy Manual QCR_SER_RSID

SER_4 AP SPOT TEST (top ↑)

4.1 Type of Test

Presumptive test for the presence of semen.

4.2 Reagents and Supplies

1) AP Spot Test Solution A
2) AP Spot Test Solution B
3) Deionized Water

4.3 Controls

4.3.1 The following controls are required to be run and recorded once per lot on each day that AP Spot is used.

1) A positive control made up of known semen.
2) A negative control consisting of Solution A and B only.

4.3.2 If a daily control fails, troubleshooting should be performed based on analyst training and experience. Continued failure should be brought to the attention of the unit supervisor and technical manager, and that lot should be removed from use in casework.

4.3.3 Requirement for lot numbers/expirations listed in case record:

4.3.3.1 AP Spot Test Solution A

4.3.3.2 AP Spot Test Solution B

4.3.3.3 Deionized Water

4.3.3.4 Known semen used for Positive Control

4.4 Individual Steps of the Procedure

4.4.1 Rubbing Method

1) Wet filter paper or swab with deionized water and rub suspected stain.
2) Add ~3 drops of AP Spot Test solution to the filter paper or swab.
3) Observe paper or swab for the presence of a color within two minutes.

4.4.2 Cutting Method

1) Take a cutting of suspected stain and place in well on plate or on a piece of filter paper.
2) Add ~3 drops of AP Spot Test solution to well or filter paper.
3) Observe well or paper for the presence of a color within two minutes.

4.5 Interpretation of Results

1) Positive: A purple color develops within two minutes.
2) Negative: No color change is observed or any other color develops besides purple within two minutes.

ATTACHMENT(S):
FBU Policy Manual QCR_SER_APSpot

SER_5 P30 DETECTION USING IMMUNOASSAY (top ↑)

5.1 Type of Test

Confirmatory test for the presence of p30, a component of semen.

5.2 Reagents and Supplies

1) Phosphate Buffered Saline (PBS)
2) p30 test kits (Seratec PSA SemiQuant or other approved device)

5.3 Controls

5.3.1 The following controls are required to be run and recorded once per lot on each day that a p30 device is used.

1) A positive control of an approximately 1:100 dilution of reconstituted semen standard or known semen.
2) A negative control consisting of saline.

5.3.2 Any testing device used that does not yield all expected internal control results is considered invalid, and the test for that sample must be re-run using a new device.

5.3.3 If a daily control fails, troubleshooting should be performed based on analyst training and experience. Continued failure should be brought to the attention of the unit supervisor and technical manager, and that lot should be removed from use in casework.

5.3.4 Requirement for lot numbers/expirations listed in case record:

5.3.4.1 p30 test kit (Seratec PSA SemiQuant or other approved device)

5.3.4.2 Phosphate Buffered Saline

5.3.4.3 Semen Standard / Known semen used for Positive Control

5.4 Individual Steps of the Procedure

1) Place a cutting of a suspected semen stain or swab into a microcentrifuge tube. If sample is a liquid, reference step 5 below.
2) Add 200µl to 750 µl of phosphate buffered saline to the tube.
3) Allow the sample to soak for approximately 30 minutes.
4) Following the extraction, remove the cutting from the tube and place the cutting into a spin basket inside the tube or in the perforated tube cap and centrifuge the sample for approximately 5 minutes at maximum speed.
5) Add approximately 200 µl of supernatant transferred to a tube from the SER_6 procedure to the sample well of the test device. If the sample is a liquid, 200 µl or a dilution of the sample may be used.
6) Observe testing device at approximately 10 minutes.

5.5 Interpretation of Results

1) Positive result: A pink line forms at the test area.
2) Negative result: No pink line forms at the test area.

NOTE: The p30 extract may be stored refrigerated for up to 5 days (from time of extraction until time of performing the test) while the analyst searches the related sperm slides prepared using the SER_6 procedure.

ATTACHMENT(S):
FBU Policy Manual QCR_SER_p30

SER_6 MICROSCOPIC IDENTIFICATION OF SPERMATOZOA (top ↑)

6.1 Type of Test

Confirmatory test for the presence of semen.

6.2 Reagents and Supplies

1) Phosphate Buffered Saline
2) X-mas Tree Stain: Nuclear Fast Red Stain and Picroindigocarmine Stain
3) Methanol or Ethanol
4) Deionized Water
5) Permount

6.3 Controls

6.3.1 Known human sperm cells previously stained with X-mas tree stain should be viewed and recorded prior to casework.

6.3.2 Requirement for lot numbers/expirations listed in case record:

6.3.2.1 Christmas Tree Stain (red and green)

6.3.2.2 Known human sperm slide used for Positive Control

6.4 Individual Steps of the Procedure

1) Place a cutting of a suspected semen stain, swab, or liquid into a microcentrifuge tube.
2) Add 200µl to 750 µl of phosphate buffered saline to the tube and extract for approximately 30 minutes.
3) During the extraction, cloth samples should be sonicated for approximately 15 minutes.
   NOTE: Analysts may use discretion for sonicating other sample types.
4) Following the extraction, remove the cutting from the tube and place into a tube basket inside the tube or in the perforated tube cap and centrifuge the sample for 5 minutes at maximum speed.
5) Remove the supernatant and place into another tube for use in p30 testing.
6) Re-constitute the pellet in approximately 10µl of phosphate buffered saline.

7) Remove all remaining sample and apply to a microscope slide.
8) Heat fix the smear to the slide by heating with a hot plate.
9) Stain the smear with one or more drops of Nuclear Fast Red Stain (Stain A) for 5 to 15 minutes.
10) Rinse the slide with deionized water to remove any excess stain.
11) Stain the smear with one or more drops of Picroindigocarmine Stain (Stain B) for 5 to 30 seconds.
12) Rinse the slide with either ethanol or methanol to remove any excess stain.
13) Allow the slide to air dry or place the slide on a hot plate and allow the slide to completely dry.
14) Using Permount in a hood, attach a cover slip to the slide.
15) View the slide microscopically (confirm spermatozoa with a 40x objective) and record the absence or presence of spermatozoa as well as the slide number (and well designation, if applicable).

6.5 Interpretation of Results

1) Positive result: Intact or partial sperm cells observed.
2) Negative result: No sperm cells observed.

ATTACHMENT(S):
FBU Policy Manual QCR_SER_Xmas

SER_7 HAIR ANALYSIS (top ↑)

7.1 Type of Test

Screening for the presence of human hair with nuclear material suitable for nuclear DNA analysis.

7.1 Equipment

Microscope

7.2 Individual Steps of the Procedure

The following macroscopic characteristics should be documented before performing a microscopic analysis:

1) Approximate color and shade
2) Hair form such as straight, curved, wavy, loose curl, tight curl, etc.
3) Approximate length to the nearest centimeter

NOTE: When analyzing a large number of hairs, macroscopic characteristics can be used when forming a sampling plan. Hairs may be grouped by the analyst based on macroscopic characteristics, and then microscopic analysis can be performed on a select number from that group. In the event that microscopic analysis shows the hairs chosen in the sampling plan from the specific group are non-human, the analyst can then decide that no further analysis is necessary on that group of hairs.

However, no opinion can be formed and applied to the entire group as to the species of origin of the hairs based on the microscopic analysis of a few hairs, and report wording should reflect this.

If a microscopic analysis is necessary, then the following characteristics shall be identified (as appropriate) **and documented in the case notes by written description or photograph**:

4) Characteristics of the medulla such as diameter with relation to the whole hair
5) Any other distinguishing characteristics (for example, apparent artificial color)
6) The presence or absence of a root
7) If the root is present, whether there is cellular material present

Based on both macroscopic and microscopic characteristics, determine whether the hair may be of human or non-human origin using the following criteria as **guidance**. Based on the written and/or photographic documentation, another qualified analyst should be able to draw the same conclusion as the examining analyst:

Factors for Determination of Hair as Human v. Non-Human

COLOR/PIGMENTATION

1. **For human hairs, color will be relatively consistent along the shaft – aside from chemical treatments.**
2. **For human hairs, pigment is typically distributed evenly or distributed toward cuticle – exception is red hair where the distribution is toward the medulla.**
3. **Animal hairs can exhibit color banding – varies along shaft**

MEDULLA

1. **Human hair medulla is typically < 1/3 of the diameter of the hair shaft and amorphous in structure**
2. **Animal hair is typically >1/3 of diameter of the hair shaft**
3. **Human hair medulla can be fragmentary (trace), interrupted (discontinuous), continuous or absent**
    a. **This isn't always consistent between hairs from one head and can vary for person**
4. **Animal hair medulla can be lattice, uniserial, multiserial (corn cob), cellular or vacuolated**

ROOT SHAPE

1. **Human hair root shape is bulbous**
2. **Animal hair root shape is characteristic for some (i.e. dog – spade, deer – wine glass)**

DIAMETER

1. **Human hair diameter variation is relatively moderate; with a shaft diameter of ~30 - 110µm with an average of ~80µm**
2. **Animal hair might have some diameter variation along shaft – distinct in some animals; diameter of ~5µm to 2mm**

SCALES

1. **Human hair has only imbricate scales**
2. **Animal hairs have varied scale structures**

8) For hairs that are determined to be non-human, no further analysis is necessary.

9) If the analyst cannot determine whether human or non-human, the analyst shall determine the hair as "inconclusive" and continue analyzing as though the hair is human, conducting any further analysis accordingly (i.e. analyze for DNA if nuclear material is present).

10) Hairs that appear to have similar characteristics macroscopically and/or microscopically may be able to be eliminated from further analysis based on circumstances of the case. Any elimination that is made and the reasons why must be documented in the case notes.

11) If a hair is to be analyzed for nuclear DNA, then it must be photographed microscopically with no less than a 10x objective prior to any destructive analysis being performed.

FBU DNA Standard Operating Procedures

The procedures for the AutoMate Express instrument (outlined in Sections 1.1 and 2.1 below) will be used for all samples types for DNA casework. If the analyst desires to use the organic extraction method (outlined in sections 1.2 and 2.2 below), the use of this method shall be approved by the Technical Manager PRIOR to analysis beginning on the sample(s). *The following table has guidelines as to the amount of sample to be taken for DNA testing. However, as each sample will be different, it is at the case analyst's discretion to use an appropriate amount of sample for testing. Before any analyst performs an action to an evidentiary item that is beyond the sample types listed below and that action is potentially destructive and irreversible, it is the responsibility of the analyst to obtain approval from the Technical Manager PRIOR to performing this action.*

Sample Type	Sample Requirement	Storage/ Preservation	DNA Origin	Special Handling Procedures	PrepFiler® Kit
Liquid Samples (Whole Blood, Saliva)	Up to 40µL	Prepare stain and store at room temperature	White blood cells, Epithelial Cells	N/A	Express
Blood stain	Up to 25mm^2 cutting	Freeze or room temperature	White blood cells	N/A	Express
Body Fluids (Saliva, Semen) on fabric	Up to 25mm^2 cutting	Freeze or room temperature	Epithelial cells, Sperm cells	N/A	Express
Body Fluids (Saliva, Semen) on swabs	Up to one swab	Freeze or room temperature	Epithelial cells, Sperm cells	N/A	Express
Chewing Gum	~ 3×3×5mm piece	Freeze or room temperature	Epithelial cells which line the mouth	Freeze sample prior to cutting to harden gum	BTA
Alternate Chewing Gum	Up to one swab	Freeze or room temperature	Epithelial cells which line the mouth	Freeze sample prior to swabbing to harden gum	Express
Tape / Envelope Flap / Stamp	~1cm^2 cutting	Freeze or room temperature	Epithelial cells which line the mouth	Cut into small pieces. Items can be removed using steam.	BTA
Alternate Tape / Envelope Flap / Stamp	Up to one swab	Freeze or room temperature	Epithelial cells which line the mouth	Swab a designated area of the item, then proceed with extraction of swab	Express
Cigarette Butt	Up to 25mm^2 cutting from around 1/2 mouth contact area	Freeze or room temperature	Epithelial cells which line the mouth	Cut paper into smaller pieces prior to placing in extraction tube	Express
Teeth	Up to 50mg powdered from a large tooth with no restoration	Freeze	Dental pulp	Clean the tooth using 1:10 bleach and rinse with deionized water. Freeze, then cut or break to expose the inner surface and remove soft tissue and pulverize a portion	BTA
Bone	Up to 50mg powdered bone	Freeze	Marrow	Clean the bone using 1:10 bleach and rinse with deionized water. Crush (or pulverize) the bone and place into extraction tube	BTA
Body Tissue	Up to 25mm^2 cutting	Freeze	Tissue cells	Mince sample prior to extraction	Express
Hair	Up to 5mm cutting from root	Room temperature	Epithelial cells from hair follicle	Wash thoroughly with 95% (or greater) ethanol and rinse with deionized water prior to extraction. If mounted, use xylene to remove mounting media.	Express
Miscellaneous Swabs	~1/2 to whole swab head	Room temperature	Various	N/A	Express
Fingernails	Up to one fingernail	Room temperature	Tissue cells	Mince sample prior to extraction, if desired. Sample can be incubated for 40 minutes up to 17 hours.	Express

DNA_1 STANDARD EXTRACTION OF DNA (top ↑)

1.1 Type of Test / Extraction Method

Isolation of DNA from all samples using the Automate Express™ Instrument and the PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits.

Reagents and Supplies

1. 1 M DTT
2. Proteinase K

Equipment

1. Mechanical Pipettors
2. ThermoMixer
3. AutoMate Express™
4. PrepFiler® *Express* and/or PrepFiler® *Express* BTA Forensic DNA Extraction Kits
5. Centrifuge

Controls

1. All extraction sets require the concurrent processing of at least one reagent blank.
2. Associated reagent blanks will be setup and evaluated in accordance with FBU QM_ 9.

Individual Steps of the Lysate Preparation for PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction

Standard Isolation of DNA using PrepFiler®® *Express*

1. Preheat the Thermomixer to 70˚C.
2. Place sample/substrate into a PrepFiler® Lysep Column/hingeless tube assembly.
3. If desired, prepare a sufficient volume of PrepFiler® lysis solution/master mix by mixing 500µL of PrepFiler® lysis buffer and 5 µL of 1M DTT per sample.
4. Add freshly prepared PrepFiler® lysis solution/master mix to each sample. Or alternatively, add 500 µL of PrepFiler® lysis buffer and 5 µL of 1M DTT individually to each sample without having to prepare a master mix as in step 3.
5. Incubate on a Thermomixer at 70˚C and 750rpm for 40 minutes.
6. Centrifuge the column/tube assembly for 2 minutes at ~10400rpm to transfer the lysate to the sample tube.
7. Remove the Lysep™ column from the sample tube.
8. If the volume of sample lysate collected in the sample tube is less than 300µL:
   a. Centrifuge the column/tube assembly for an additional 5 minutes.
   b. If the volume is still less than 300µL, add PrepFiler® Lysis Buffer to bring the lysate volume to ~300µL.
9. If a pellet is present in the sample tube, transfer the clear lysate to a new PrepFiler® sample tube.
10. If you observe any salt precipitation, heat the lysate to 37°C until the precipitate goes back into solution, then use a pipette to mix the sample lysate. Do not load any sample tube that contains

precipitate on the AutoMate *Express*™ Instrument, or instrument crash, tip clogging, or filter wetting may occur.

11. Set-up and run the AutoMate, as follows:

a. Power on the instrument. (Optional: Clean the piercing units at this time using only alcohol.)

b. Press start and open the door.

c. Load and insert the cartridge rack ensuring that the magnetic particles are suspended by mixing the cartridges.

d. Load and insert the tip and tube rack with samples.

i. Row S (fourth row): Load with PrepFiler® samples tubes containing the lysate.

ii. Row T2 (third row): Load with AutoMate Express tips inserted into tip holders.

iii. Row T1 (second row): Leave empty.

iv. Row E (first row): Load with labeled PrepFiler® elution tubes, with caps open and secured.

e. Close the instrument door and press the enter arrow. Select the appropriate script (PrepFiler® Express [1] or PrepFiler® Express BTA [2]).

f. Press Start.

g. At the end of the run, press the enter arrow to return to the Main Menu and then open the instrument door.

h. Close the caps on the sample elution tubes. All volumes should be at ~50µL.

i. Remove the racks from the instrument. Store the sample tubes appropriately and discard the used reagent cartridges, tips and tubes into a designated, non-bleach containing PrepFiler® biohazard waste bin.

j. Clean the piercing units on the instrument using alcohol only.

NOTE: No bleach is to be used on the instrument or racks (toxic fumes may be produced).

NOTE: Steps taken to any sample must also be performed on an associated reagent blank, according to QAS Standard 9.5.3.

NOTE: If the instrument door needs to be opened during a run, PAUSE the run BEFORE opening the door.

NOTE: Do not Crosslink (UV) the Lysep columns.

Standard Isolation of Bone and Teeth DNA using PrepFiler® *Express* BTA

1. Preheat the ThermoMixer to 56˚C.
2. Clean the bone or tooth to remove any adhered tissue.
3. Prepare a uniform bone or tooth powder using standard laboratory procedures.
4. Transfer approximately 50mg of powdered bone or tooth into a new PrepFiler® Bone and Tooth Lysate Tube.
5. If desired, prepare a sufficient volume of PrepFiler® lysis solution master mix by mixing:

   220µL of PrepFiler® BTA Lysis Buffer
   3µL of 1 M DTT
   7µL of Proteinase K
6. Add 230µL of freshly-prepared PrepFiler® BTA lysis solution/master mix to each sample. Or alternatively, add each of the reagents listed in step 5 individually to each sample without having to prepare a master mix as in step 5.
7. Screw the cap on the PrepFiler® Bone and Tooth Lysate Tube, vortex it for 5seconds, then centrifuge it no longer than 5 seconds.
8. Incubate on a Thermomixer at 56˚C and 1100rpm for at least 2 hours (no more than 18 hours).
9. Centrifuge the tube for 90 seconds at ~10400rpm.
10. Transfer the clear lysate (no sediment) to a new PrepFiler® sample tube.
11. If the volume of sample lysate collected in the sample tube is less than 150µL, then add PrepFiler® BTA Lysis Buffer to bring the lysate volume to 150µL.
12. Set-up and run the AutoMate as follows:
    a. Power on the instrument. (Optional: Clean the piercing units at this time using only alcohol).
    b. Press start and open the door.
    c. Load and insert the cartridge rack ensuring that the magnetic particles are suspended by mixing the cartridges.
    d. Load and insert the tip and tube rack with samples.
       i. Row S (fourth row): Load with PrepFiler® samples tubes containing the lysate.
       ii. Row T2 (third row): Load with AutoMate Express tips inserted into tip holders.
       iii. Row T1 (second row): Leave empty.
       iv. Row E (first row): Load with labeled PrepFiler® elution tubes, with caps open and secured.
    e. Close the instrument door and press the enter arrow. Select the appropriate script (PrepFiler® Express [1] or PrepFiler® Express BTA [2]).

f. Press Start.

g. At the end of the run, press the enter arrow to return to the Main Menu and then open the instrument door.

h. Close the caps on the sample elution tubes. All volumes should be at ~50µL.

i. Remove the racks from the instrument. Store the sample tubes appropriately and discard the used reagent cartridges, tips and tubes into a designated, non-bleach containing PrepFiler® biohazard waste bin.

j. Clean the piercing units on the instrument using alcohol only.

NOTE: No bleach is to be used on the instrument or racks (toxic fumes may be produced).

NOTE: Steps taken to any sample must also be performed on an associated reagent blank, according to QAS Standard 9.5.3.

NOTE: If the instrument door needs to be opened during a run, PAUSE the run BEFORE opening the door.

NOTE: Do not Crosslink (UV) the Lysep columns.

Standard Isolation of Adhesive sample types DNA using PrepFiler® *Express* BTA

1. Prepare the sample using standard laboratory procedures listed above or in consultation with the Technical Manager.
2. Preheat the ThermoMixer to 56˚C.
3. Place sample into a column/tube assembly.
4. If desired, prepare a sufficient volume of PrepFiler® lysis solution/master mix by mixing 220µL PrepFiler® BTA Lysis Buffer, 3µL of 1M DTT, and 7 µL of Proteinase K.
5. Add 230 µL of freshly prepared PrepFiler® lysis solution/master mix to each sample. Or alternatively, add 220 µL of PrepFiler® lysis buffer, 3 µL of 1M DTT, and 7 µL of Proteinase K individually to each sample without having to prepare a master mix as in step 4.
6. Incubate on a Thermomixer at 56˚C and 750rpm for approximately 40 minutes.
7. Centrifuge the tube for 2 minutes at ~10400rpm to transfer the lysate into the sample tube. Remove the Lysep column containing the substrate from the sample tube and retain, if necessary.
8. Transfer the clear lysate to a new PrepFiler® Sample Tube.
9. If the volume of sample lysate collected in the sample tube is less than 150µL, then add PrepFiler® BTA Lysis Buffer to bring the lysate volume to 150µL.
10. Set-up and run the AutoMate as follows:
    a. Power on the instrument. (Optional: Clean the piercing units at this time using only alcohol.)

b. Press start and open the door.

c. Load and insert the cartridge rack ensuring that the magnetic particles are suspended by mixing the cartridges.

d. Load and insert the tip and tube rack with samples.

   i. Row S (fourth row): Load with PrepFiler® samples tubes containing the lysate.

   ii. Row T2 (third row): Load with AutoMate Express tips inserted into tip holders.

   iii. Row T1 (second row): Leave empty.

   iv. Row E (first row): Load with labeled PrepFiler® elution tubes, with caps open and secured.

e. Close the instrument door and press the enter arrow. Select the appropriate script (PrepFiler® Express [1] or PrepFiler® Express BTA [2]).

f. Press Start.

g. At the end of the run, press the enter arrow to return to the Main Menu and then open the instrument door.

i. Close the caps on the sample elution tubes. All volumes should be at ~50µL.

j. Remove the racks from the instrument. Store the samples appropriately and discard the reagent cartridges, tips, and tubes into a designated, non-bleach containing PrepFiler® biohazard waste bin.

k. Clean the piercing units on the instrument using alcohol only.

NOTE: No bleach is to be used on the instrument or racks (toxic fumes may be produced).

NOTE: Steps taken to any sample must also be performed on an associated reagent blank, according to QAS Standard 9.5.3.

NOTE: If the instrument door needs to be opened during a run, PAUSE the run BEFORE opening the door.

NOTE: Do not Crosslink (UV) the Lysep columns.

FBU DNA Standard Operating Procedures

1.2 Type of Test / Extraction Method

Organic isolation of DNA from all non-differential samples.

1.2.1 Reagents and Supplies

1. Stain Extraction Buffer
2. Proteinase K
3. Phenol/Chloroform/ Isoamyl Alcohol (PCI)
4. TE^{-4} Buffer
5. Microcon YM-100 (tubes and filters)

1.2.2 Equipment

1. Mechanical Pipettors
2. Thermometers

1.2.3 Controls

1. All extraction sets require the concurrent processing of at least one reagent blank.
2. Associated reagent blanks will be setup and evaluated in accordance with FBU QM_ 9.

1.2.4 Individual Steps of the Organic Extraction Procedure

1.2.4.1 <u>Standard Isolation of DNA</u>

1. Place sample into a tube.
2. Add 400µL Stain Extraction Buffer.
3. Add 10µL Proteinase K.
4. Vortex briefly and spin down.
5. Incubate at 56°C (+/- 1°C) on heat block for 6 to 24 hours (for questioned samples) or 2 to 24 hours (for known samples).
6. Place sample into spin basket and microcentrifuge at 10,000 – 15,000 rpm for ~5 min.
7. Remove the spin basket. Retain the sample if necessary.
8. In the fume hood, add 500µL Phenol/Chloroform/Isoamyl Alcohol to the extract.
9. Vortex ~5 – 10 sec to achieve a milky emulsion.
10. Microcentrifuge ~5 minutes at 10,000 – 15,000 rpm.

2) **Purification of Isolated DNA by Microcon Ultrafiltration**

1. Insert the filter into each tube.
2. Without disturbing the interface, transfer the aqueous (top) layer from the microcentrifuge tube to the filter.
3. Spin the Microcon in a microcentrifuge at ~2300 rpm (500 rcf) for ~20 minutes. If necessary, continue to spin at ~2300 rpm for 5 – 10 min increments until almost all the liquid has appeared to have passed through the filter.
4. *Optional Step:* Add 200 – 400µL of TE^{-4} to the filter. Then spin the Microcon in a microcentrifuge at ~2300 rpm (500 rcf) for ~20 minutes. If necessary, continue to spin at ~2300 rpm for 5 – 10 min increments until almost all the liquid has appeared to have passed through the filter

   NOTE: If it appears the filter may be clogged/saturated (i.e. the volume is not passing through) the sample may be transferred to an additional Microcon device in order to facilitate the filtration – remembering to treat the associated reagent blank in the same manner.
5. Collect the concentrated DNA by inverting the filter into a second Microcon tube.
6. Spin the inverted filter in a microcentrifuge at ~3300 rpm (1000 rcf) for 3 minutes.
7. The approximate volume of extract is then determined and recorded on the extraction worksheet. The extract must be transferred to a new microcentrifuge tube to prevent evaporation.
   a. For Reagent Blanks: The volume collected must be at or below a working volume of 30µL.
   b. For Sample Extracts: At this point, the volume of the extract should be approximately 30µL, to allow for both autosomal and male testing unless the sample is a low level touch-type sample; in such case, the sample extract can be ~15µL. Ensure the associated regent blank is at an equal or lesser volume. However, with some robust samples (i.e. apparent neat blood stains) the sample volume can exceed the working volume of 30µL, based on analyst discretion and experience.
8. If the reagent blank or sample extract is below the working volume of 30µL, additional TE^{-4} can be added to bring the sample/reagent blank to this volume, if needed. Reagent blanks will not be diluted above a volume of 30µL.

   NOTE: If inhibition is observed in a sample *after* the quantitation step, additional PCI or Microcon washes of the extract and its associated reagent blank using the procedure outlined above may be performed to help rid the sample of the inhibitor. Additionally, at analysts' discretion, an additional PCI and Microcon washes may be performed to the extract and its associated reagent blank *prior to* the quantitation

step due to extract color, sample type, etc. If the quantitation value obtained for the sample is lower than desired, the sample may also be concentrated using a Microcon filter device or the DNA Speedvac in order to reach the desired amplification target. These concentrations are to take place AFTER quantitation. **STEPS TAKEN TO ANY SAMPLE IN THIS MANNER MUST ALSO BE PERFORMED ON AN ASSOCIATED REAGENT BLANK**, according to the QAS Standard 9.5.3.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_Automate_Ecell_Digest_Buffer
FBU Policy Manual QCR_DNA_Automate_EXTReagents
FBU Policy Manual QCR_DNA_OrgEXTReagents
FBU Policy Manual QCR_DNA_OrgSAReagents
FBU Policy Manual QCR_DNA_PCI
FBU Policy Manual QCR_DNA_TE-4
FBU Policy Manual DNA_AutomateExtract
FBU Policy Manual DNA_OrganicExtract
FBU Policy Manual DNA_Lots

REFERENCE(S):

FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
Life Technologies PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits User Guide
Life Technologies Automate Express Instrument User Guide

DNA_2 DIFFERENTIAL EXTRACTION OF DNA (top ↑)

2.1 Type of Test / Extraction Method

Isolation of DNA from sexual assault samples using the Automate Express™ Instrument and the PrepFiler® *Express* Forensic DNA Extraction Kits.

2.1.1 Reagents and Supplies

1. 1 M DTT
2. Proteinase K
3. E-Cell Digest Buffer
4. Autoclaved Water

2.1.2 Equipment

1. Mechanical Pipettors
2. ThermoMixer
3. AutoMate Express™
4. PrepFiler® *Express*™ Forensic DNA Extraction Kit
5. Centrifuge

2.1.3 Controls

1. All extraction sets require the concurrent processing of at least one reagent blank.
2. Associated reagent blanks will be setup and evaluated in accordance with FBU QM_ 9.

2.1.4 Individual Steps of the Lysate Preparation for PrepFiler® *Express*™ Forensic DNA Extraction

2.1.4.1 Differential Isolation of DNA

1. Preheat the Thermomixer to 56˚C.
2. Place swab/substrate into a spin tube and add 395µL E-Cell Digest buffer (10mM Tris-HCl, 10mM EDTA, 50mM NaCl, 2% SDS, pH 7.5) and 5µL of 20mg/mL Proteinase K solution.
3. Vortex to mix and incubate at 56ºC for 1 hour.
4. After removing from Thermomixer, spin down samples briefly to eliminate condensation on tube caps.
5. Place swab/substrate in spin basket and spin at ~12,200rpm for 5 min.
6. Save or discard swab/substrate.
7. Without disturbing the sperm pellet, transfer the supernatant (epithelial fraction [EF]) into a separate tube and process for DNA isolation as described below in step 14. Be careful not to disturb the sperm pellet during transfer; if necessary, up to 50µL of supernatant can remain in the tube with the pellet.
8. Wash the sperm pellet 3 times with autoclaved water:
   a. Add 500µL autoclaved water to the sperm suspension.
   b. Vortex and spin at ~12,200rpm for 5 min.
   c. Without disturbing the sperm pellet, remove and discard as much of the supernatant as possible during each wash step. Be careful not to disturb the sperm pellet; if necessary, up to 50µL of the supernatant can remain in the tube with the pellet after the final wash.
9. If desired, prepare a sufficient volume of PrepFiler® lysis solution/master mix by mixing 500µL of PrepFiler® lysis buffer and 5µL of 1M DTT per sample.
10. Add 500µL of **freshly prepared** PrepFiler® lysis solution/master mix to the sperm pellet from step 8c. Or alternatively, add 500 µL of PrepFiler® lysis buffer and 5 µL of 1M DTT individually to the sperm pellet from step 8c without having to prepare a master mix as in step 9.
11. *Optional Step:* Add lysis/sperm pellet solution to a new tube to avoid evaporation during incubation.
12. Vortex and incubate on a Thermomixer at70ºC and 750rpm for 40 minutes.
13. Transfer the sperm lysate (~500µL) to a hinge-less sample tube labeled as sperm fraction (SF).
14. For the EF collected from step 7, perform the following:
    a. Aliquot 50 µL of EF from step 7 above into a hingeless sample tube.
       NOTE: If more than 50 µL of EF is to be processed, based on case circumstances, concentrate the EF and use 50 µL. (This is to maintain desired salt concentration for binding DNA to magnetic particles.)

The remaining EF volume (and its associated blank volume) may be stored in the

refrigerator until the case is closed in case there is a need to analyze the EF further.

b. To this aliquot, add 450 µL of PrepFiler® lysis buffer (without DTT – cells are already lysed).

NOTE: This step is to bring the salt concentration to desired level for DNA binding.

c. Proceed directly to automated extraction run.

NOTE: Both EF (after conditioning with PrepFiler® lysis buffer) and SF can be processed simultaneously on AutoMate (in one instrument run) since the protocol for isolation of DNA is identical.

15. Set-up and run the AutoMate as follows:

a. Power on the instrument. (Optional: Clean the piercing units at this time using only alcohol.)

b. Press start and open the door.

c. Load and insert the cartridge rack ensuring that the magnetic particles are suspended by mixing the cartridges.

d. Load and insert the tip and tube rack with samples.

i. Row S (fourth row): Load with PrepFiler® samples tubes containing the lysate.

ii. Row T2 (third row): Load with AutoMate Express tips inserted into tip holders.

iii. Row T1 (second row): Leave empty.

iv. Row E (first row): Load with labeled PrepFiler® elution tubes, with caps open and secured.

e. Close the instrument door and press the enter arrow. Select the appropriate script (PrepFiler® Express [1] or PrepFiler® Express BTA [2]).

f. Press Start.

g. At the end of the run, press the enter arrow to return to the Main Menu and then open the instrument door.

h. Close the caps on the sample elution tubes. All volumes should be at ~50µL.

i. Remove the racks from the instrument. Store the sample tubes appropriately and discard the used reagent cartridges, tips and tubes into a designated, non-bleach containing PrepFiler® biohazard waste bin.

j. Clean the piercing units on the instrument using alcohol only.

NOTE: No bleach is to be used on the instrument or racks (toxic fumes may be produced).

NOTE: Steps taken to any sample must also be performed on an associated reagent blank, according to QAS Standard 9.5.3.

NOTE: If the instrument door needs to be opened during a run, PAUSE the run BEFORE opening the door.

NOTE: Do not Crosslink (UV) the Lysep columns.

2.2 Type of Test / Extraction Method

Organic isolation of DNA from sexual assault samples.

2.2.1 Reagents and Supplies

1. TNE (Tris/NaCl/EDTA)
2. Proteinase K
3. Phenol/Chloroform/Isoamyl Alcohol (PCI)
4. TE^{-4}
5. 20% Sarkosyl
6. 0.39M DTT
7. Autoclaved Water
8. Sperm Wash Buffer
9. Microcon YM-100

2.2.2 Equipment

1. Mechanical Pipettors
2. Thermometers

2.2.3 Controls

1. All extraction sets require the concurrent processing of at least one reagent blank.
2. Associated reagent blanks will be setup and evaluated in accordance with FBU QM_ 9.

2.2.4 Individual Steps of the Differential Organic Procedure

2.2.4.1 <u>Differential Isolation of DNA</u>

1. Place sample into a tube.
2. Add:
   a. 400µl TNE
   b. 75µl H_2O
   c. 25µl 20% Sarkosyl
   d. 5µl Proteinase K

   TOTAL: 505µl

3. Vortex briefly and spin down.
4. Incubate at 37°C on heat block for ~2 hours
5. Place sample into spin basket and microcentrifuge at 10,000 to 15,000 rpm for ~5 min.
6. Remove the spin basket. Retain the sample if necessary.
7. Remove the supernatant fluid without disturbing the pellet and place in a new tube.
8. Wash the cell pellet by adding 500µL of sperm wash buffer, vortex briefly, and microcentrifuge at 10,000 – 15,000 rpm for ~5 minutes.
9. Remove and discard the supernatant fluid without disturbing the pellet.
10. Repeat previous two steps for a total of three washes.
11. To the pellet add:
    a. 150µl TNE
    b. 150µl H_2O
    c. 50µl 20% Sarkosyl
    d. 40µl 0.39 M DTT
    e. 10µl Proteinase K

    TOTAL: 400µl
12. Vortex briefly and spin down.
13. Incubate at 37°C on heat block for 2 to 24 hours.
14. In the fume hood, add 500µL Phenol/Chloroform/Isoamyl Alcohol to the extracts.
15. Vortex ~5 – 10 sec to achieve a milky emulsion.
16. Microcentrifuge ~5 minutes at 10,000 – 15,000 rpm.

2.2.5 Purification of Isolated DNA by Microcon Ultrafiltration

1. Insert the filter into each tube.
2. Without disturbing the interface, transfer the aqueous (top) layer from the microcentrifuge tube to the filter.
3. Spin the Microcon in a microcentrifuge at ~2300 rpm (500 rcf) for ~20 minutes. If necessary, continue to spin at ~2300 rpm for 5 – 10 min increments until almost all the liquid has appeared to have passed through the filter.
4. *Optional Step:* Add 200 – 400µL of TE^{-4} to the filter. Then spin the Microcon in a microcentrifuge at ~2300 rpm (500 rcf) for ~20 minutes. If necessary, continue to spin at ~2300 rpm for 5 – 10 min increments until almost all the liquid has appeared to have passed through the filter

   NOTE: If it appears the filter may be clogged/saturated (i.e. the volume is not passing through) the sample may be transferred to an additional Microcon device in order to facilitate the filtration – remembering to treat the associated reagent blank in the same manner.
5. Collect the concentrated DNA by inverting the filter into a second Microcon tube.

6. Spin the inverted filter in a microcentrifuge at ~3300 rpm (1000 rcf) for 3 minutes.
7. The approximate volume of extract is then determined and recorded on the extraction worksheet. The extract must be transferred to a new microcentrifuge tube to prevent evaporation.
   a. For Reagent Blanks: The volume collected must be at or below a working volume of 30µL.
   b. For Sample Extracts: At this point, the volume of the extract should be approximately 30µL to allow for both autosomal and male testing unless the sample is a low level touch-type sample; in such case, the sample extract can be ~15µL. Ensure the associated regent blank is at an equal or lesser volume. However, with some robust samples (i.e. apparent neat blood stains) the sample volume can exceed the working volume of 30µL, based on analyst discretion and experience.
8. If the reagent blank or sample extract is below the working volume of 30µL, additional TE^{-4} can be added to bring the sample/reagent blank to this volume range if needed. Reagent blanks will not be diluted above a volume of 30µL.

NOTE: If inhibition is observed in a sample *after* the quantitation step, additional PCI or Microcon washes of the extract and its associated reagent blank using the procedure outlined above may be performed to help rid the sample of the inhibitor. Additionally, at analysts' discretion, an additional PCI and Microcon washes may be performed to the extract and its associated reagent blank *prior to* the quantitation step due to extract color, sample type, etc. If the quantitation value obtained for the sample is lower than desired, the sample may also be concentrated using a Microcon filter device or the DNA Speedvac in order to reach the desired amplification target. These concentrations are to take place AFTER quantitation. **STEPS TAKEN TO ANY SAMPLE IN THIS MANNER MUST ALSO BE PERFORMED ON AN ASSOCIATED REAGENT BLANK**, according to the QAS Standard 9.5.3.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_Automate_Ecell_Digest_Buffer
FBU Policy Manual QCR_DNA_Automate_EXTReagents
FBU Policy Manual QCR_DNA_OrgEXTReagents
FBU Policy Manual QCR_DNA_OrgSAReagents
FBU Policy Manual QCR_DNA_PCI
FBU Policy Manual QCR_DNA_TE-4
FBU Policy Manual DNA_AutomateExtract
FBU Policy Manual DNA_OrganicExtract
FBU Policy Manual DNA_Lots

REFERENCE(S):

FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
Life Technologies PrepFiler® *Express* and PrepFiler® *Express* BTA Forensic DNA Extraction Kits User Guide
Life Technologies Automate Express™ Instrument User Guide

DNA_3 DNA QUANTITATION (top ↑)

3.1 Type of Test

Quantify the approximate amount of amplifiable human DNA or human male DNA in a forensic sample.

3.2 Reagents and Supplies

1) TE^{-4} Buffer w/ Glycogen
2) Quantifiler™ Human Kit or Quantifiler™ (Y) Human Male Kit
3) 96-well optical reaction plate
4) Optical Adhesive Cover

3.3 Equipment

1) Mechanical Pipettors
2) AB 7500

3.4 Controls

3.4.1 Each quantitation plate requires a QNC.

3.4.2 If the QNC displays a signal, then the Technical Manager will be notified and deem if the plate is acceptable or if it may need to be re-setup and re-run.

3.4.3 Associated reagent blanks will be setup and evaluated in accordance with FBU QM_ 9.

3.5 Individual Steps of the Procedure

3.5.1 Preparing the Reactions

1) Prepare standards according to QCR_DNA_QUANTSTNDS, if necessary.
2) Vortex the Quantifiler™ Human or Quantifiler™ (Y) Human Male Primer Mix and spin down.
3) Swirl the Quantifiler™ PCR Reaction Mix gently (no vortex).
4) Prepare the Master Mix by combining the appropriate volume of each component in a tube, vortex and spin down.
5) Dispense 23 µl of Master Mix into the appropriate wells of the plate.
6) Pipette 2 µl of each standard, sample, and associated controls into the appropriate well.
7) Seal the plate with an Optical Adhesive Cover.
8) Spin down the plate until all air bubbles at the bottom of the wells are removed.

3.5.2 Running the Reaction

1) Place the 96-well reaction plate in the 7500 instrument.
2) Launch the 7500 System Software and set-up plate document.
3) Make sure that the following values are selected:
   - Assay: Absolute Quantification (Standard Curve)
   - Container: 96-well Clear
   - Template: Blank Document (or a pre-designed template if available/appropriate)
   - Operator: Analyst's Name/Initials
   - Comments Section: Analysts may list case numbers if not in plate name – not required
   - Default Plate Name: Can copy comments section or enter an appropriate plate name (i.e. initials and case number, batch number, or date).
4) In the Plate Tab view, enter the standards and samples. Only if multiple cases are batched together on a plate is the case number or batch identifier (reagent blanks) required in the sample name.
5) Ensure the appropriate detectors are selected and the thermal cycling parameters are correct.
5) Save file in the appropriate folder.
6) Click on **Start** to begin run.

3.5.3 Analyzing the Results

1) Omit unused wells. Empty wells should NOT be omitted prior to running the assay.
2) Run the analysis ensuring the R^2 value is >0.98.
3) Outliers within the standard curve can be removed leaving at least one replicate of each standard (when using optional duplicate standard plate setup). One outlier within the standard curve can be removed when using single standard setup. Contact the Technical Manager if the need for removing more than one standard arises.

3.5.4 Evaluation of Samples for Genetic Analysis

1) Check samples and controls for possible inhibition or improper amplification. If observed, the sample may need to be re-quantitated.
2) Any sample that has a low or zero DNA quantitation that would require that the full volume of the extract to be taken on for amplification must have a total volume of extract not in excess of the associated reagent blank from the extraction step. Further concentration of any samples that have an extraction volume in excess of the associated reagent blank through additional process steps such as the use of Microcons or SpeedVacs may be done at this point along with any associated reagent blanks.

3) If the quantitation results for any sample are above 50ng/µl, the sample must be diluted and re-quantified.

NOTE: If it is determined that the quantitation value obtained for the sample is lower than desired, the sample may be concentrated using a DNA Concentrator (SpeedVac) in order to reach the desired amplification target. The same steps must be taken with an associated reagent blank that are taken with the samples to comply with QAS Standard 9.5.3. The concentration of any sample and reagent blank will be documented in the case record.

The following steps will be used when operating the DNA Concentrator:

1) Clean the DNA concentrator with a 1:10 bleach solution prior to use.
2) Turn the DNA concentrator on and choose the appropriate heat setting (high, medium, or low).
3) Places the sample(s) and associated reagent blank(s) in the DNA concentrator with the lids of the tubes open, ensuring that they are balanced.
4) Run the DNA concentrator for an amount of time required to dry down the sample volumes to the appropriate volume or less. If necessary, add the required volume of TE^{-4} to bring the sample volumes back up to appropriate volume for amplification.
5) Clean the DNA concentrator with a 1:10 bleach solution.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_QUANTKIT
FBU Policy Manual QCR_DNA_QUANTSTNDS
FBU Policy Manual DNA_Quant
FBU Policy Manual DNA_Lots

DNA_4 PCR AMPLIFICATION USING IDENTIFILER® PLUS (top ↑)

4.1 Type of Test

STR amplification of DNA samples

4.2 Reagents and Supplies

1) AmpF*l*STR Identifiler® Plus Kit
2) TE^{-4}

4.3 Equipment

1) Mechanical Pipettors
2) Thermal cycler

4.4 Controls

4.4.1 Associated reagent blanks and controls will be setup and evaluated in accordance with FBU QM_9 and CASE_4.

4.5 Individual Steps of Procedure

1) Thaw (if necessary), vortex, and centrifuge briefly the AmpF*l*STR Identifiler® Plus Kit Master Mix and AmpF*l*STR Identifiler® Plus Kit Primer Set.
2) Prepare the Master Mix for all samples to be amplified by adding the appropriate volumes of reagents to a tube.
3) Vortex and spin down.
4) Dispense 15µl of Master Mix into each tube/well.
5) Add the appropriate volume of sample and/or TE^{-4} to each tube/well. A target range of 0.5ng to 1.0ng based on sample type/quantitation/analyst discretion is recommended. If the target range of 0.5ng to 1.0ng cannot be reached, based on a low quantity of DNA present in the sample, amplification of the maximum volume (10 µl) of sample will be performed.
6) Place the tubes/plate in the thermal cycler and start the amplification program titled "ID Plus" per the 28 cycle internal validation.
7) Remove tubes/plate from the thermal cycler.
8) Amplified products shall be stored refrigerated or frozen and must be protected from light. Amplified products may not be used after two weeks from the date of amplification without

prior Technical Manager approval. Upon completion of the Technical Review of the case, these products should be discarded.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_AMPKIT
FBU Policy Manual DNA_PCRSetup
FBU Policy Manual DNA_Norm
FBU Policy Manual DNA_Lots

DNA_5 PCR AMPLIFICATION USING YFILER™ (top ↑)

5.1 Type of Test

STR amplification of DNA samples for male component

5.2 Reagents and Supplies

1) AmpF*l*STR Yfiler™ Kit
2) TE^{-4}

5.3 Equipment

1) Mechanical Pipettors
2) Thermal cycler

5.4 Controls

5.4.1 Associated reagent blanks and controls will be setup and evaluated in accordance with FBU QM_9 and CASE_4.

5.5 Individual Steps of the Procedure

1) Gently vortex the AmpF*l*STR PCR Reaction Mix, AmpF*l*STR Yfiler™ Primer Set, and AmpliTaq Gold DNA Polymerase and spin down.
2) Prepare the AmpF*l*STR Yfiler™ Master Mix for all samples to be amplified by adding the appropriate volumes of reagents to a tube.
3) Vortex and spin down.
4) Dispense 15 µL of Master Mix into each tube/well.
5) Add the appropriate volume of sample and/or TE^{-4} to each tube/well. A target range of 0.5ng to 1.0ng based on sample type/quantitation/analyst discretion is recommended. If the target range of 0.5ng to 1.0ng cannot be reached, based on a low quantity of DNA present in the sample, amplification of the maximum volume (10 µl) of sample will be performed.
6) Place the tubes/plate in the thermal cycler and start the amplification program titled "Yfiler" per the 30 cycle internal validation.
7) Remove tubes/plate from the thermal cycler.
8) Amplified products shall be stored refrigerated or frozen and must be protected from light. Amplified products may not be used after two weeks from the date of amplification without

prior Technical Manager approval. Upon completion of the Technical Review of the case, these products should be discarded.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_AMPKIT
FBU Policy Manual DNA_PCRSetup
FBU Policy Manual DNA_Norm
FBU Policy Manual DNA_Lots

DNA_6 3500 GENETIC ANALYSIS OF IDENTIFILER® PLUS AND YFILER™ SAMPLES (top ↑)

6.1 Type of Test

Obtaining a genetic profile with the 3500 Genetic Analyzer from DNA amplified with Identifiler® Plus or Yfiler™.

6.2 Reagents and Supplies

1) 96-well optical reaction plate
2) 96-well septa and plate retainer
3) Identifiler® Plus or Yfiler™ ladder
4) Hi-Di™ Formamide or equivalent
5) GS600 LIZ® v2.0 Internal Size Standard
6) Anode and Cathode Buffer

6.3 Equipment

1) Mechanical Pipettors
2) Genetic Analyzer (3500)

6.4 Individual Steps of the Procedure

6.4.1 Preparing Samples, Controls, and Ladders for 3500 Genetic Analysis

1) Prepare a master mix of Hi-Di™ Formamide and GS600 LIZ® v2.0 as follows:
   - (number of samples + 2) x 8.5 µL Hi-Di™ Formamide
   - (number of samples + 2) x 0.5µL GS600 LIZ® v2.0.
2) Vortex and spin down.
3) Aliquot 9 µl of master mix into each well with a sample.
4) Aliquot 9 µl of master mix or Formamide only into empty wells in the columns which contain samples in an injection set.
5) Add 1 µl of sample/controls/ladder to each well.

6) Seal plate with septa and spin down if necessary.
7) Heat denature each sample at ~95°C for approximately 3 minutes.
8) Snap cool each sample at ~0°C for approximately 3 minutes.
9) Preheat the oven to 60ºC from the main dashboard, if desired.

6.4.2 Loading of Samples onto the 3500 Genetic Analyzer

1) Snap the plate retainer over the sample plate and plate base.
2) Place the plate assembly onto the auto sampler.

6.4.3 Electrophoresis of Samples with the 3500 Genetic Analyzer

1) Open the **3500 Series Software** and wait for the service console to start all application services.
2) Click "**Create New Plate**" in the Dashboard.
3) In the Define Plate Properties screen, enter the plate name for your plate and ensure that "**96**" is selected for the Number of Wells, "**36cm**" is selected for Capillary Length, "**HID**" for Plate Type and "**POP4**"is selected for the Polymer Type. You can save the plate at this point if you would like.
4) Click **Assign Plate Contents** and fill in the appropriate Sample Names, Sample Types, Assay, Filename Convention and Results Group (you can use the plate view or table view to enter this information).

   NOTE: Sample names must reflect the biologist's initials, case number, item number, and kit type. Controls must reflect the biologist's initials, case number and/or date, control name, and kit type. Ladders must reflect "Ladder" and kit type.

   NOTE: Do not use special characters (other than an underscore) as they are not recognize by the software and can cause the software to be unable to find the plate records in the datastore.
5) Another option is to import a plate from a template. To do this, after you click on "**Create a New Plate**", enter the plate name then click on assign plate contents. In the next screen select the "**Import**" button and import the plate from the appropriate location, then assign the Sample Types, assays, File Name Conventions, and Results Group, as appropriate.
6) At this point you can either Click "**View Plate Grid Report"** and print this view for your case file or use the 3500 setup worksheet for your case file.

   Note: Adjust the view of the table before printing by zooming out, as needed; the view that you see on the screen is what will print.
7) Save the plate and click on "**Link Plate for Run.**"
8) Click on "**Create Injection List**."
9) From here, you can setup any duplicate injections as necessary. Any re-injection that needs to be performed can be done from this screen only while the run is going.
10) Click on the "**Start Run**" button.

NOTE: You can also modify the injection list before you start the run from the "Preview Run" screen. To duplicate an injection (same parameters), select an injection, then click the icon with two downward pointing arrows. To use a different protocol for a replicate injection, specify a re-injection in the "Monitor Run" screen after you start the run.

NOTE: Run reagent blanks according to the most sensitive injection conditions per FBU QM_9.

NOTE: All samples and reagent blanks must be evaluated at an injection time of 15 seconds for the peak stochastic threshold (PST). The analyst has the option to inject using the most sensitive injection times during the initial set up of the instrument.

ATTACHMENT(S):

FBU Policy Manual QCR_DNA_Formamide
FBU Policy Manual DNA_3500Setup (optional)
FBU Policy Manual QC_3500_Maint_Log
FBU Policy Manual DNA_Lots

DNA_7 GENEMAPPER ID-X ANALYSIS OF STR DATA (top ↑)

7.1 Type of Test

The GeneMapper ID-X (GMID-X) software analyzes the data collected by the instrument to size and quantify the DNA fragments.

7.2 Individual Steps of the Procedure

1) Launch the **GeneMapper ID-X** application.
2) Add sample files to the Project Window.
3) Select the Appropriate Sample Type
   - Allelic ladders as "Allelic Ladder"
   - Forensic samples as "Sample"
   - Reagent blanks and negative controls as "Negative Control"
   - Positive control as "Positive Control"
4) Select the Appropriate Analysis Method

   For the **OSBI Casework_ID+_15 sec_v1_8-2013** and **OSBI Casework_ID+_24 sec_v1_8-2013**, the parameters should be defined as follows:

   Allele Tab:

   Bin Set: AmpFLSTR_BINS_v3x

   "Use marker-specific stutter ratio if available" is selected

   Minus Stutter Distance for Tetra From: 3.25 To: 4.75

   All other values should be 0.0

   Peak Detector Tab:

   Sizing: Partial Sizes Start Size: 80 Stop Size: 400

   Peak Detection Algorithm: Advanced

   Smoothing: Light

   Baseline Window: 51 pts

   Size Calling Method: Local Southern Method

   Peak Amplitude Thresholds:

Analytical Thresholds		
Identifiler Plus		
Dye	**15 seconds**	**24 seconds**
Blue	100	150
Green	190	220
Yellow	200	250
Red	250	300

Min. Peak Half Width: 2 pts

Polynomial Degree: 3

Peak Window Size: 15 pts

Peak Start: 0.0

Peak End: 0.0

Use normalization, if applicable is checked

For the **OSBI Casework_Yfiler_15 sec_v1_8-2013** and **OSBI_Casework_Yfiler_24 sec_v1_8-2013**, the parameters should be defined as follows:

Allele Tab:

Bin Set: AmpFLSTR_BINS_v3x

Use marker-specific stutter ratio if available is selected

Minus Stutter Distance for:

Tri	From: 2.25	To: 3.75
Tetra	From: 3.25	To: 4.75
Penta	From: 4.25	To: 5.75
Hexa	From: 5.25	To: 6.75

All other values should be 0.0

Peak Detector Tab:

Sizing: Partial Sizes Start Size: 80 Stop Size: 400

Peak Detection Algorithm: Advanced

Smoothing: Light

Baseline Window: 51 pts

Size Calling Method: Local Southern Method

Peak Amplitude Thresholds:

Analytical Thresholds		
Yfiler		
Dye	**15 seconds**	**24 seconds**
Blue	280	390
Green	290	360
Yellow	220	290
Red	230	300

Min. Peak Half Width: 2 pts

Polynomial Degree: 3

Peak Window Size: 15 pts

Peak Start: 0.0

Peak End: 0.0

Use Normalization, if applicable is checked

5) Select the Appropriate Panel
   - Identifiler® Plus: Identifiler_Plus_v1.2X
   - Yfiler™: Yfiler_v1.2X
6) Select the Appropriate Size Standard
   - Identifiler® Plus and Yfiler™: Use GS600_LIZ+Normalization_(80-400)
7) Analyze the samples. Save the project using, at a minimum, your initials, the case number and a descriptor such as run date, Yfiler, IDPlus or number/other identifier (for example, project # or injection #) in this format: "**ABC 14-1234 other descriptor**".
8) If a sample is ran multiple times (i.e. re-amplification, re-setup) and is present in the same project multiple times, the samples shall be clearly labeled during the 3500 sample data entry so they can be distinguished in the sample column in the downstream GMID-X project (i.e. Item 1A_reamp, Item 1A_resetup).
9) Generally each case will have its own separate GMID-X project. A "batch" project is allowed, with prior Technical Manager approval; however, during the review portion of cases in this prior approved batch project, the technical reviewer is responsible only for reviewing the samples in the case which they are reviewing along with the case's associated ladders and controls.
10) Evaluate the data in accordance with FBU CASE_5.

CASE_1 Notes (top ↑)

1.1 General Case Notes

1.1.1 Case notes will be taken using applicable fields of the BEAST matrix panels. A worksheet will be generated from the BEAST matrix panel information using the BEAST worksheet generator for incorporation into the final case record. Any DNA worksheets will be retained electronically in the BEAST using the Adobe Portfolio PDF program (portfolio). The portfolio will be organized using different headers for each applicable "section" of the case file, as follows, to facilitate a review-friendly case file: BEAST notes worksheet, extraction worksheet, quantitation setup worksheet, quantitation results, normalization worksheet, amplification setup worksheet, genetic analysis setup worksheet, technical notes, electropherograms, results worksheet, statistical calculations, and corrections performed as a result of review. Additional folders are acceptable if case/analysis circumstances warrant, as necessary (i.e. STRbase microvariant confirmation). The portfolio is considered for the Forensic Biology Units to be the "final case record". Any corrections required to the portfolio discovered during technical and/or administrative review will be tracked using a "corrections folder" in the portfolio document or thoroughly documented in the BEAST routing history. Any changes made to the report will need to be documented in the BEAST either through the BEAST routing history or through the narrative (may include e-mail communication). NOTE: For serology cases, if all information is contained within the BEAST matrix panels, the "final case record" for this case can be either the BEAST-generated worksheet or a portfolio can be created (analyst discretion). All DNA cases will use the portfolio case record organization.

1.1.2 Include a description of the outer evidence packaging for each item of evidence.

1.1.3 Dates of analysis and item numbers will be documented in the case notes in accordance with OSBI CSD QP 16.2 – Contents of Case Records.

1.1.4 Overall visual description of the item to include size or reference to size, color(s), and condition (as applicable).

1.1.5 A photograph of the item may be used in lieu of a written description outlined above.

1.1.6 If hairs and/or debris were collected from the item, if applicable.

1.1.7 The size/amount of any biological material testing positive from the item. This may be documented with a photograph (with scale, if possible).

1.1.8 The use of any stereomicroscopic and/or microscopic search unless required by the test performed (i.e., Takayama, Cell Search).

1.1.9 The use of an alternative/ultraviolet light source.

1.1.10 Any other pertinent information necessary to document the analysis of the evidence.

1.1.11 If stains are produced from a liquid sample that has been submitted, the number and size or volume of stains made.

1.1.12 In instances where the entire sample shall be consumed in testing, refer to OSBI CSD QP 6.1 –

Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance and QP 16.2 – Contents of Case Records for appropriate documentation.

For ALL major crimes (persons crimes) and for property crimes with a listed or known subject, prior written authorization allowing consumption of the item(s) of evidence from the District Attorney's Office will be obtained and maintained in the case record. For property crimes with no listed subject or with an unknown subject, prior written authorization from the District Attorney is NOT required prior to consuming the item of evidence for analysis.

In cases where samples or DNA extracts are received from another agency or laboratory for testing, documentation from the District Attorney (or other prosecuting agency) granting permission of consumption shall be obtained from the requesting agency or laboratory and maintained in the OSBI case record.

1.1.13 Lot numbers and expiration dates of reagents used in testing. If multiple lot numbers of the same reagent are used, the analyst must document which lot number was used for which day of testing or which lot was used for the controls and/or samples.

1.1.14 Serology controls must be tested prior to/or in conjunction with evidence samples. Results of tested controls must be documented in the analyst's notes. In the event controls do not yield expected results, conclusions may not be reported for the associated tests.

1.2 **Batching:** The OSBI Forensic Biology Units may batch cases together. If cases are batched together, the following parameters should be maintained:

1.2.1 Personal identifying information from each case (suspect's names or victim's names) should be maintained only in the appropriate case file.

1.2.2 Shared analysis worksheets with multiple case numbers present shall include the case number for the appropriate case file. It is not required to specifically indicate the case number (i.e. underline or circle since highlighting won't replicate when imaged), but this is recommended as it aids in the review process. Each electronic case file will have the case number and the examining analyst's initials, at minimum, indicated on each page either in a header or elsewhere on each page of the file.

1.2.3 All case numbers comprising a batch should be clearly represented in the case notes for each case file.

1.2.4 All information/data for controls associated with the amplification kit used in each respective case must be included in the case file.

1.2.5 With which case the reagent blank(s) was/were stored (if applicable).

1.3 **Use of Technicians:** The OSBI Forensic Biology Units may utilize trained technicians in the laboratory for various laboratory functions, including assisting analysts with casework. If technicians are utilized or if another analyst is acting in the capacity of a technician, the following parameters will be maintained with respect to casework. The analyst must clearly identify the technician(s) and the steps performed by the technician(s) in the case notes for each case by having the technician(s) initial/sign the appropriate pages in the case file either on the hard copy or electronically.

1.4 **DNA Case Notes Specific Requirements**

1.4.1 A description of the approximate amount of material consumed in testing such that the amount of sample and extract remaining can be extrapolated from the notes.

1.4.2 DNA worksheets as applicable should be completed. The following worksheets are required if performed:

- DNA_OrganicExtract, DNA_AutoMateExtract – DNA Extraction Worksheets
- DNA_Lots – DNA Lot Number Worksheet
- DNA_Quant – DNA Quantitation Worksheet
- DNA_PCRSetup – DNA Amplification Setup Worksheet
- DNA_3500Setup (or 3500 Plate Grid Report plate record with spectral listed)

The use of non-policy and/or additional worksheets in the case record is discouraged unless the worksheet adds clear value to the case.

1.4.2.1 Technical Notes should be utilized to account for all injections as well as a verification of all parameters, size standards, and ladders. In lieu of a technical notes worksheet, documentation of injections may be listed elsewhere in the notes. Technical notes may be used to explain any deviations from protocol, any occurrences resulting in re-injections or other additional analysis, or to record reasons for decisions made about interpretation or terminating further analysis.

1.4.3 With the exception of known artifacts, any required documentation of artifacts should be included in the case notes. This documentation may include a “GeneScan” view or a zoom view of electropherograms.

1.4.4 At a minimum, all electropherograms used in reporting data including the associated controls shall be included in the case notes.

1.4.5 Occasionally, samples must be re-injected, re-setup, re-amplified, or re-extracted. Each electropherogram generated that is included in the case file must be clearly labeled such that a reviewer can identify from which sample it came and in what order it was generated. An unlabeled electropherogram shall signify an original injection performed with the default injection time of 15 seconds. All other electropherograms must contain labeling indicating their injection order – the injection time is listed in the sample naming in GMID-X.

1.4.6 At a minimum, any consumption photos, the 7500 quantitation data, genetic analyzer run folder(s), and all GeneMapper ID-X projects should be available for technical review and placed onto the

DNA server for storage.

- GMID-X projects are to contain all injections of samples and controls for each batch/case. The raw data is available in the raw data tab for each injection so it is retained with each sample/control in the GMID-X project. Any injections that are not used for interpretation/reporting are to be accounted for in the "Technical Notes" portion of the case record. The location of the retained GMID-X projects is to be listed in the case record (by statement in the BEAST case record - i.e. "photos, 7500 data, 3500 data and GMID-X project(s) retained on the DNA server").

1.4.7 A Results Table must be included in the case file. Analysts may report DNA profiles using either phenotypes or genotypes on a sample by sample basis. Samples with two alleles in the results for Yfiler amplifications at the DYS385 locus will be reported using a comma.

ATTACHMENT(S):

FBU Policy Manual QM_Abbrev
FBU Policy Manual DNA_OrganicExtract
FBU Policy Manual DNA_AutoMateExtract
FBU Policy Manual DNA_Lots
FBU Policy Manual DNA_Quant
FBU Policy Manual DNA_PCRSetup
FBU Policy Manual DNA_3500Setup (optional)

REFERENCE(S):

OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

CASE_2 Digital Photography (top ↑)

2.1 Use of Digital Images

2.1.1 Digital imagery and documentation of limited samples shall be handled according to OSBI CSD QP 16.2 – Contents of Case Records as well as the discipline specific requirements present in this section.

2.1.2 If an image shall become **the only record of an item of evidence**, this image shall be sufficiently clear to accurately represent the item to subsequent viewers. For example, prior to consumption of the item in testing or if the analyst feels that image(s) is/are essential for interpretation (i.e. blood spatter), the image should be archived (i.e. BEAST image vault, computer hard drive, DNA server) prior to proceeding to any subsequent analysis of the evidence.

2.1.3 Digital images of consumptive evidence should have a unique identifier or name following its transfer to a computer for archival purposes. The image should be re-named so that it contains sufficient information to allow easy correlation of the image file to the evidence or test results it represents. An example for a photo of a hair would be "10-1234 1 Hair".

2.1.4 At a minimum, the case number, item number, and/or description of the image should be in the image file name.

2.1.5 The original images of consumptive evidence should be retained unaltered. If enhancements are necessary, they may be performed on copies of the original images. All steps of the enhancement shall be properly documented and the analyst must be able to recreate the enhancement.

2.1.6 If the only copy of an image is placed within the LIMS system, it should be stored in the image vault and verified.

2.1.7 If the evidence shall be available in essentially unaltered form for later examination and the image is obtained as an aid in note taking, the images do not have to be archived prior to proceeding to any subsequent analysis of the evidence.

2.1.8 Images that become a part of the analyst's case notes may be annotated during the course of the examination to show particular areas of interest.

2.1.9 All images, with the exception of images of outer packaging and images taken with the aid of a microscope or stereoscope, should, if possible, include the case number, item number, date, initials and a scale in the image.

2.1.10 Images that are taken to document the state in which an item is received should be taken prior to any alteration or testing of the evidence.

REFERENCE(S):
OSBI CSD Quality Manual

CASE_3 Serology (top ↑)

Homogenizing Samples received for Sexual Assault Analysis (SAMPLING PLAN):

If a sample is received for analysis in a liquid form (i.e. urine/aspirate for seminal fluid testing), the sample will be vortexed and then spun down to pellet any potential cellular material present in the sample before testing. The sample will be tested by the same procedures as outlined in SER_5 and SER_6 above, as applicable.

3.1 Blood Analysis

NOTE: Analysts may use sample selection for testing of blood stains for heavily stained items. The documentation must clearly show that sample selection was performed such that a reviewer shall know that the number of stains tested does not equal the number of stains actually present on the item.

3.1.1 If a stain yields a presumptive blood negative result, no further testing on that stain is required unless case circumstances and/or analyst discretion warrants additional analysis.

3.1.2 If an item yields a confirmatory blood negative result with no prior presumptive test result, analysts may perform presumptive testing after-the-fact based on analyst discretion.

3.1.3 If an item yields a presumptive blood positive result, further testing may or may not be performed based on case circumstances (limited sample, etc.)

3.2 Sexual Assault Analysis

3.2.1 AP Spot Test may be used to facilitate the screening of large/heavily stained items for sexual assault evidence excluding intimate items or underwear.

3.2.2 A sampling plan may be utilized on heavily stained items to reduce the number of stains to be tested; however, with analyst discretion, additional stains may need to be tested if the results from these samples are all negative. Additional samples may need to be added to the sampling plan and tested until a positive p30 or cell search result is obtained or all stains have been subjected to confirmatory testing. However, any positive results obtained during testing may only be applied to the stains tested and cannot be the basis of an opinion about any other stains which have not been tested.

NOTE: If terminating testing of an item after AP Spot test negative results without using sample selection to test using p30/sperm testing, approval should be obtained from the Technical Manager and available in the case file.

3.2.3 If an item yields a sperm negative result with no prior presumptive test result, analysts should perform p30 testing after-the-fact. In addition, p30 may be conducted in conjunction with cell search.

3.2.4 Regardless of p30 testing results, cell search must be conducted.

3.2.5 If an item results in a positive p30 or cell search result, any smear slides present with the case do not need to be analyzed but should be retained with the evidence.

3.2.6 Faint p30 positive results on oral swabs only require further DNA analysis based on analyst discretion or if case circumstances warrant further analysis.

3.3 Hair Analysis

3.3.1 Due to the nature of hair transfer, the OSBI Forensic Biology Units will not perform hair examinations on cases where individuals have routinely or recently had close contact or access to common areas (living quarters, vehicle, etc.). If other probative evidence exists in a case, no hair examination will be performed unless case circumstances dictate otherwise. (Example: If semen is identified in a rape kit, typically hairs present in the rape kit will not be examined.)

3.3.2 The order in which the hairs are examined between evidence samples and known (reference) samples is up to the individual analyst based on the case circumstances. However, the examination of evidence samples and known samples must be separated by time and/or space.

REFERENCE(S):
OSBI CSD Quality Manual

CASE_4 DNA Analysis (top ↑)

4.1 Swabs of items from the same area/staining (i.e. vaginal swabs, bloodstain from door jamb) may be combined for the purposes of DNA analysis regardless of serology results. For example, two vaginal swabs with differing results (swab A - p30 positive, sperm negative and swab B - p30 and sperm positive) may be combined for the purposes of DNA analysis, if necessary.
Swabs that are not clearly indicated as from the same area/staining (or if that determination cannot be made) will not be combined for DNA analysis.

4.2 In the event that there is evidence of peaks below the analytical threshold and above the limit of detection (LOD), an evaluation of those peaks should be done to determine the value of the information that would be obtained with further analyses. During this evaluation, the needs of and results from the CODIS Unit should be kept in mind. For example, if the peaks below analytical threshold would make a locus heterozygous and could then be used for statistical calculations or provide information that would be useful for CODIS purposes if they were higher than the analytical threshold, then attempts to bring the peak heights above the analytical threshold with increased injection times may need to be attempted. Additionally, if many of the peaks in a profile are below the stochastic threshold such that statistical information would be limited, the DNA target should be evaluated to determine if re-amplification with more template DNA would provide better results. If the increase in amplification target or injection time would cause other interpretation problems that may result from off-scale data, then no further analysis may be necessary.

4.3 In the case of some mixtures, such as profiles obtained from intimate samples or clothing, it is not uncommon to observe a background profile from the source of the intimate sample or the wearer of the clothing. Two examples of this would be incomplete separation of the epithelial fraction of a semen stain into the sperm fraction (or vice versa) or the background profile of someone known to be wearing a particular article of clothing. In these instances, it would not be necessary to re-inject, re-setup, or re-amplify a sample if not all the peaks are above threshold. Results inconsistent with a background profile must be handled according to the first requirement above.

4.4 All forensic samples that result in no profile above threshold must be evaluated to verify these results. If a thorough evaluation for the presence of the primer peak and robust internal lane standard (ILS) peaks reveals the sample injected properly, the sample does not need to be re-injected, as a repeat or increased injection time will not bring peaks above the threshold, according to information obtained during an OSBI FBU internal validation.
Re-amplifying the sample may be necessary if a quantitation value was obtained which may happen with an extremely degraded sample.

4.5 If non-concordant results occur, the profile should be re-injected to obtain concordant results with one of the previous injections. If no result occurs with a sample obtaining a value during quantitation, the sample may need to be evaluated by re-injecting or re-amplifying the sample.

4.6 All reagent blanks and controls will be analyzed in the same manner as forensic samples.

4.7 <u>Monitoring for Contamination</u>

- All amplified samples shall be evaluated for potential contamination. Although not always readily apparent like controls, contamination shall be monitored through sample data analysis. For example, a forensic questioned sample with a very low quantitation value or a zero quantitation value yielding an off-scale profile could be an indication of a sample switch. Another example: a known reference sample yielding a mixture could be an indication of contamination.
- Signal observed in reagent blanks during quantitation will not be considered contamination unless any called peaks above threshold are obtained.
- Possible peaks below threshold may not be considered contamination in the absence of any called peaks.
- Any time an evaluation of controls yields unexpected results, the control shall be re-injected to verify the result. If the re-injection is acceptable, then the control shall be considered valid. If the re-injection is not acceptable, the incident will be brought to the attention of the Technical

Manager and the Supervisor and handled according to OSBI CSD QP 13 – Non-Conforming Work.

4.8 If possible contamination is observed the Technical Manager and Supervisor will be notified immediately. After a thorough evaluation of the data by the Technical Manager, if contamination is confirmed, the procedure in OSBI CSD QP 13 – Non-Conforming Work will be followed. If it is determined by the Technical Manager that there is no significant impact on the data due to the possible peak(s) below threshold, the data may be reported.

- If the contaminant profile is not attributed to samples in the case or batch of cases being analyzed or to OSBI employees, the profile will be forwarded to the CODIS Unit for evaluation for entry into the Contaminant Index (SDIS only). The CODIS Administrator (or designee) will evaluate these profiles to determine eligibility for entry into CODIS.

4.9 Paternity Cases

- Paternity cases should be processed analyzing the alleged father, alleged mother and offspring. Paternity case requests without a full trio of individuals will be accepted and worked on a case-by-case basis in consultation with the Technical Manager.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBU Casework Standard Operating Procedure

CASE_5 DNA Interpretation Guidelines (top ↑)

5.1 Interpretation of results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. However, it is important that the laboratory develops and adheres to minimum criteria for interpretation of analytical results. These criteria were developed with maximum input from analysts and are based on validation studies, literature references, and casework experience.

The purpose of these guidelines is to establish a general framework and outline the minimum requirements to ensure interpretations are made as objectively as possible, are consistent from analyst to analyst, and are scientifically supported by the analytical data from the samples and associated controls.

5.2 Tools used for interpretation:

- Confirmation of correct results from all associated controls (5.2.1)
- Evaluation of Allelic Peaks (5.2.2) including:
  - Allele designations given by the analysis software (GeneMapper ID-X).
  - Consideration of the peak heights and peak height ratios at each locus and across the entire profile
- Consideration of possible artifacts (5.2.3)
- Thresholds (5.2.4)
- Interpretations and Comparisons (5.2.5)
- Type of Profiles (5.2.6)

5.2.1 Confirmation of correct results from all associated controls

DNA typing results should be "verified and interpreted" (*SWGDAM Interpretation Guidelines)* by reviewing: software-generated information, internal size standards, ladders, positive amplification controls, negative amplification controls, reagent blanks, and samples.

5.2.1.1 Software Generated Information

- Correct Spectral
- Correct Panel
- Correct Size Standard
- Correct Genotyping Parameters (Analysis Method)

5.2.1.2 Review of Internal Size Standard and Ladders

- The ILS performed as expected and is present in all ladders, controls, and samples.
- All ladders selected for comparison to controls and samples performed as expected with all alleles sized and labeled correctly.

5.2.1.3 All profiles will be evaluated to ensure no extraneous or unexplained peaks are present within the analysis range of 80-400bp for Identifiler® Plus and Yfiler™.

5.2.1.4 **Review of Positive Amplification Controls, Negative Amplification Controls, and Reagent Blanks**

- The positive and negative amplification controls and all reagent blanks will be verified to ensure that the results obtained are acceptable.
- The correct profile must be obtained for the positive amplification control.
- The negative amplification control and reagent blanks(s) must have no peaks indicating the presence of contamination per QM_9.

5.2.2 Evaluation of Allelic Peaks

5.2.2.1 Peak Height Ratios

- When a locus displays two alleles in a single source sample, the allele with the lower peak height generally displays at least 50% of the peak height of the higher peak at the locus.
- In a mixture profile, the relative peak heights may be in any relative contribution.
- For Identifiler® Plus, the peak height ratio should generally be above 50% (exceptions can be CSF1PO, D2S1338, D18S51, and FGA and these should have a ratio above 40%).
- Keep in mind that peak height balance is affected by the amount and quality of template DNA available, so lower template samples may experience peak height balance of less than these general percentages.
- The technical reviewer must agree with determinations outside these criteria. If there is discrepancy, the Technical Manager should be consulted.
- Regardless of peak height, peaks determined to be the result of stutter, pull-up, lack of non-template nucleotide addition (minus A), elevated baseline, spikes or background will not be designated as true alleles.

5.2.2.2 Off-Ladder Alleles

- No additional analysis is required for virtual alleles; the GMID-X allele call will be used.

5.2.2.3 Off-Ladder Alleles - Locus Designation

- When an off-ladder (OL) allele is observed between loci, and one neighboring locus appears to be homozygous while the other is heterozygous, the OL allele most likely is a sister allele paired with the locus that appears to display homozygosity.

- Other OL allele designations between loci shall be handled on a case-by-case basis, in consultation with the Technical Manager.

5.2.2.4 Off-Ladder Alleles - Allele Designation

OL alleles will be assigned an allele value according to the following:

- Designation of the alleles should refer to the total number of full repeats.
- Designation of incomplete repeat motifs should include the number of complete repeats, and separated by a decimal point, the number of base pairs in the incomplete repeat. For example, allele 9.3 at TH01 has 9 complete repeats and one incomplete repeat of 3 base pairs.

5.2.2.5 Microvariants

- Determine the base pair size of the microvariant peak, and then compare the base pair size of the microvariant to the base pair size of the two bracketing ladder peaks.
- Determine the size difference between the microvariant and the smaller of the ladder peaks and round to the nearest whole number. For example, if a green off-ladder allele peak sizes at 238.39 bases and the "36" allele of the D21S11 ladder is 236.32 bases, the peak in question shall be labeled as "36.2" because the difference is 2.07, or 2 bases in length.

5.2.2.6 Off-Ladder Allele Calculating

- Determine the base pair size of your off-ladder allele.
- Determine the size difference between the off-ladder allele and the nearest ladder peaks. Round the size to the nearest whole number and then divide by the repeat size for that locus. Add or subtract this number to the nearest ladder allele (as appropriate) to determine your off-ladder allele designation. For example, in D3S1358, the smallest ladder allele is 109.89 base pairs, designated as "12." Your off-ladder allele is 101.81 base pairs. 109.89 – 101.81 is 8. The size repeat for D3S1358 is 4 base pairs. 8/4 = 2, 12 - 2 = 10. Your off-ladder allele would be designated as "10."
- Any off-ladder allele more than 8 base pairs away from the nearest ladder allele should be calculated with caution, since tetranucleotide repeats do not always size exactly 4 base pairs apart.
- All calculations should be documented on the electropherograms with sizes labeled or in the Technical Notes of the case record.
- All off-ladder and microvariant alleles, excluding virtual alleles, shall be researched (user's manual, published literature, or NIST STRbase variant allele

reports at (http://www.cstl.nist.gov/strbase/var_tab.htm) to determine if the observed allele value has been previously reported. Compare the assigned value of the off-ladder allele found in the sample in question to the list of other known alleles for that locus.

- If such an allele has been observed in the population, the allele shall be accepted and reported as indicated below. The allele will be renamed in the GMID-X project as determined.
- If the allele has not been observed, and is present in multiple samples, the allele shall be accepted and reported as indicated below.
- If the allele has not been observed, and the allele is only present in one sample, that sample will be re-amplified if possible. If the sample cannot be re-amplified due to limited material, that allele shall be called inconclusive and the locus containing that allele shall not be used in any statistical calculations.

- Those alleles, which fall outside the ladder for any locus, shall be reported as the determined allele value/< (smallest ladder peak) or > (greater than smallest ladder peak). From the example above, the allele would be reported as "10/<12". The allele will be renamed in the GMID-X project as "10" and on the PDF of the electropherogram as determined ("10/<12"). Alleles which fall outside the ladder will be entered on the CODIS Data Entry Form as <X or >X, as applicable.
- All verified microvariant alleles should have the reference documented in the technical notes or on the electropherograms.

5.2.2.7 Trisomy

- If a profile is suspected to have trisomy at a locus, the sample should be re-amplified if possible to verify this event. If the trisomy is observed in multiple samples, no further analysis is necessary.
- The trisomy can be reported; however, the locus shall not be used in any single source genotype frequency calculations.

5.2.3 Considerations of Possible Artifacts - The following are defined as artifacts by the Forensic Biology Units and will be designated as follows. Peaks that do not fit any of the following designations will be considered extraneous and will be handled on a case by case basis in consultation with the Technical Manager.

5.2.3.1 PCR Products – Stutter

- Marker specific stutter ratios (n-4) shall be utilized based on internal validation data and the manufacturer's recommendations. If marker specific stutter ratios are unavailable (for example n+/- 2), analysts shall rely on training, experience and validation data for the determination whether a peak is categorized as stutter.

- The presence of n+4 stutter may present issues when analyzing the minor component of a mixture profile with high concentrations of DNA for the major component, based on data obtained during an OSBI internal validation of interpretation thresholds. The data obtained from the OSBI validation will be used when interpreting data affected by n+4 stutter (it is available as an attachment to the Forensic Biology Unit Policy Manual).
- With higher than recommended amounts of template DNA, stutter may sometimes be called as a peak by GMID-X software by exceeding the marker-specific stutter ratio. Analysts must evaluate the profile as a whole to determine whether the peak is a true allele or a stutter peak.
- Generally, when the peak height of a stutter peak exceeds the OSBI stutter expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele (SWGDAM Guidelines, Section 3.5.8.2). Analysts will rename confirmed stutter peaks as "ST". Analysts may document the stutter peak ratio and the marker-specific ratio. The technical reviewer must agree with all stutter determinations.
- If a sample appears to be from more than one person, the entire profile must be carefully considered before determining a peak to be stutter rather than a true allele.

5.2.3.2 PCR Products – Minus A

- Analysts may place the affected sample(s), PC, NC, and associated reagent blank(s) on the thermal cycler for an additional 10 minute extension at 60°C. Any samples subjected to the additional extension should be re-setup and analyzed on the genetic analyzer.
- Alternatively, if interpretations are not affected by the minus A, analysts may rename minus A as "-A" in the GMID-X project.
- If samples with minus A are interpreted, analysts shall not utilize any quantitative aspects of interpretation (such as stutter and peak height ratios). The technical reviewer must agree with all minus A determinations.

5.2.3.3 PCR Products – Trailing Primer Peak

- Analysts may rename trailing primer peaks as "TP". The technical reviewer must agree with all trailing primer peak determinations.

5.2.3.4 Kit Specific Artifacts – Identifiler® Plus

- Reproducible kit specific artifacts for Identifiler® Plus have been reported by the manufacturer as outlined below:
- There is a kit specific artifact that has been acknowledged by Life Technologies. This artifact is in the blue dye and is associated with peaks in the size standard. This artifact should be below the analytical threshold, but may appear above the

limit of detection. This could impact evaluations of negative controls, negative samples, and samples with potentially low level DNA.

- If observed above threshold, analysts may rename artifacts as "ART". Analyst may document the known artifact location and that the artifact was renamed. The technical reviewer must agree with all renamed artifacts.

5.2.3.5 Kit Specific Artifacts – Yfiler™

- Reproducible non-calling region artifacts have been observed in the green (88 bp), yellow (80 and 95 bp) and red (80 bp) dyes.
- If observed above threshold, analysts may rename artifacts as "ART". Analyst may document the known artifact location and that the artifact was renamed. The technical reviewer must agree with all renamed artifacts.

5.2.3.6 Analytical Artifacts – Instrument Spikes

- In the event an instrument spike interferes with the analysis of an electropherogram or interferes with an allele call, the sample in question should be re-injected to obtain a clean electropherogram.
- Analysts will rename called spikes as "SP". The technical reviewer must agree with all spike determinations.

5.2.3.7 Analytical Artifacts – Raised Baseline

- In the event a raised baseline interferes with the analysis of an electropherogram or interferes with an allele call, the sample in question shall be re-injected to obtain a clean electropherogram.

5.2.3.8 Instrument Limitations – Off-Scale Peaks

- An examination of the OS (Off-Scale) PQV in the Genotypes tab is acceptable for determining whether a peak is off-scale.
- If the off-scale peak is not causing any interpretation problems, the analyst may use the injection as long as off-scale peak(s) are documented.
- If the off-scale peak is causing any interpretation problems, the analyst should attempt to alleviate the problem by re-injecting the sample at a lower injection time.
- If the off-scale peak is causing any interpretation problems and the reduction of injection time either resulted or will result in the loss of allelic information, the analyst shall select the most informative profile.
- If samples with peaks flagged as off-scale are interpreted, analysts should not utilize any quantitative aspects of interpretation (such as stutter and peak height ratios) from the artificial peak height value assigned to the off-scale peak at that location.
- Depending on the type or level of interpretation problems observed from off-scale data, analysts may have to re-amplify the sample at a lower amplification target to obtain reportable results.

5.2.3.9 Instrument Limitations – Pull-up

- Analysts will rename confirmed pull-up peaks as "PU". The technical reviewer must agree with all pull-up determinations. Alternatively, the analyst may choose to re-inject the sample at 7 seconds if this will not adversely affect the profile obtained.
- Pull-up may be observed with peaks near the linear detection limit of the instrument but technically not off-scale, with the presence of off-scale peaks, or as a result of a poor spectral on the instrument.

5.2.3.10 Introduced into the Analysis Process – Dye Blobs

- In the event a dye blob interferes with the analysis of an electropherogram or interferes with an allele call, the sample in question should be re-injected to obtain a clean electropherogram.
- Dye blobs observed above threshold outside of allele calling ranges shall be deemed acceptable without re-injection.
- Analysts will rename dye blobs as "DB." The technical reviewer must agree with all dye blob determinations.

5.2.4 Thresholds

5.2.4.1 The **peak stochastic threshold** is the relative fluorescence unit (rfu) level for an amplification kit that has been established through internal validation procedures to alert analysts of the possibility of encountering stochastic issues with allelic peaks that have peak heights below this threshold.

- These effects are due to a low amount of template DNA present which affects the quality of the amplification process.
- Peaks observed below the peak stochastic threshold (PST) that do not appear to have a paired allelic peak cannot be assumed to be homozygous. Dropout of a potential paired allelic peak must be assumed for statistical calculations. Peak height ratios of paired allelic peaks below PST may also be lower than normal. Any interpretation of mixtures with peaks below the PST should be done carefully and in the most conservative manner possible because if there are alleles present between the analytical and stochastic threshold, other alleles may have dropped out. Y-STR mixtures with the highest peak below the peak stochastic level will be called "inconclusive" or in some instances may be interpreted with caution and in consultation with the Technical Manager.
    - Known samples with peaks below the PST can be reported as genotypes (8,8) instead of phenotypes (8) as long as the technical reviewer agrees with this determination.

Stochastic Threshold	
Identifiler Plus	**Yfiler**
950	2000

NOTE: No stochastic threshold exists for longer injection times; therefore, all samples and controls must first be analyzed with a 15 second injection time.

- Samples may be classified as displaying stochastic effects if:
  - The profile has unpaired allelic peaks that have peak heights above the analytical threshold, but below the stochastic threshold.
  - The profile has lower peak height ratios than would be expected between two paired allelic peaks.
  - The profile has extreme sloping.
  - The profile has allelic dropout.

  NOTE: If any profile has resolution issues or any other abnormality, the analyst will consult with the Technical Manager before reporting the data.

5.2.4.2 Analysts may declare potential for allelic dropout to have occurred based on either:

5.2.4.2.1 There also appears to be allelic dropout occurring at loci with smaller base pair ranges (i.e. if single peak at D21S11 that is above stochastic threshold may be designated as partial if there is also dropout occurring at D8S1179, D3S1358, D19S433, or Amelogenin).

5.2.4.2.2 The locus in question has a clearly defined peak above the instrument baseline, but below the limit of detection that cannot be attributed to an artifact. It is suggested for this instance that further analysis (additional extractions) be performed on this sample, if possible, to distinguish this peak further.

- If a peak that appears below the **analytical threshold but above limit of detection** is believed to be the result of an allele, additional analysis (i.e. re-inject at longer injection time or re-amp at higher target) should be attempted in order to bring as much data as possible above the analytical threshold. However, in some instances this may not be practical due to higher peak heights of other alleles in a sample. Any peaks above the analytical threshold must be evaluated to determine if the peak is an allele or present due to an artifact. Criteria found within this policy should be utilized to determine if a peak is an artifact.

Analytical Thresholds		
Identifiler Plus		
Dye	15 seconds	24 seconds
Blue	100	150
Green	190	220
Yellow	200	250
Red	250	300

Analytical Thresholds		
Yfiler		
Dye	15 seconds	24 seconds
Blue	280	390
Green	290	360
Yellow	220	290
Red	230	300

5.2.4.3 On a case-by-case basis, consideration of peaks between the **limit of detection** and the analytical threshold may assist in interpretation of the results. Example: to confirm exclusion.

Limits of Detection		
Dye	Identifiler Plus	Yfiler
Blue	70	180
Green	100	160
Yellow	110	130
Red	140	140

- The Technical Reviewer must agree with use of alleles below analytical threshold. If there is disagreement, the Technical Manager will be consulted.
- Any peaks observed with heights below the limit of detection will not, in all likelihood, be able to be elevated to a peak height of relevance with increased injection times.
- Re-amplification with a higher target may be necessary for these peaks to provide any potential useful information, or additional analysis with another method, such as Y-STR analysis may be necessary. Any further attempt to assess peaks below the limit of detection will be up to the discretion of the analyst and the needs of the case.

5.2.4.4 Any peaks below the **instrument baseline threshold** cannot be reliably distinguished from peaks associated with random baseline noise. These peaks most likely do not provide useful information and are not to be interpreted.

Instrument Baseline Thresholds	
Blue	20
Green	30
Yellow	50
Red	70

5.2.5 INTERPRETATIONS AND COMPARISONS

ATTENTION: DNA typing results for forensic unknown samples should be verified and interpreted, to the extent reasonably possible, prior to performing comparisons to reference samples

NOTE: When performing interpretations and comparisons, keep in mind the potential for allele sharing and additive effects of such sharing; this can affect not only allele peak heights, but also stutter. **Also, please keep in mind, non-paternity cases dealing with relatedness should be interpreted with extreme caution and in consultation with the Technical Manager.**

5.2.5.1 Interpretation of single source samples with all peaks above the peak stochastic threshold (PST)

- If the sample appears to be from a single source, and two alleles are detected at a locus, the analyst can be confident that the DNA profile at the locus is complete, regardless of the stochastic threshold.
- Likewise, if only one allele is detected at a locus and this allele is above the stochastic threshold, the analyst can be confident that there is no allelic dropout.

5.2.5.2 Interpretation of single source samples with peaks below the peak stochastic threshold (PST)

- If the sample appears to be from a single source and only one allele is detected at a locus that is below the stochastic threshold, the analyst may not be confident that the DNA profile is complete at that locus.
- If the sample is a known reference sample and only one homozygous locus is below stochastic threshold with no indications of a peak below analytical threshold and all other heterozygous loci are well balanced, the profile can be assumed to be complete.
- In such instances where there is not confidence that the profile is complete, the analyst must indicate that this single peak is below the stochastic threshold and appropriate statistical calculations will be performed on this locus using OSBI STATS – dropout “yes”.
- If the profile is to be considered complete for statistical purposes (i.e. other questioned sample(s) display the same profile with all results above PST), reasoning for this must be reflected in the technical notes and agreed on by the technical reviewer of the case.

- **Partial profiles (single source) must have interpretable results at a minimum of four loci for interpretation.**

5.2.5.3 <u>Interpretation of samples that appear to be from more than one source:</u>

- The entire profile must be considered when making a determination of whether a profile is a mixture. The presence of more than two peaks or imbalanced peak heights may not, by themselves, constitute the profile being a mixture.
- Based on the OSBI in-house mixture study of three-, four-, and five-person mixtures, an analyst cannot accurately identify the number of contributors to a mixture; rather, analysts will interpret DNA profiles as a whole based on the number of peaks present at each locus and can make a statement about the *minimum* number of contributors to a mixture profile in their reports (i.e. at least three contributors for a profile with five peaks at one or more locus/loci). Peaks below analytical threshold but above the limit of detection will be considered toward the maximum allele count at a locus.
- Analysts will be allowed to interpret and report multi-person mixtures of DNA profiles obtained from samples consisting of a maximum count of six alleles at a single locus based on the results of the OSBI in-house mixture study. This generally equates to a mixture of at least three people, but could be a four- or five-person mixture based on the OSBI in-house mixture study results. Indistinguishable mixtures with seven or more alleles at a single locus will be deemed as "not suitable for interpretation" due to their high levels of complexity and use report wording as described in CASE_7.7.9.
- If possible, determine if the mixture is a major/minor or indistinguishable, as outlined below:
    - Option 1 – Mixture where the peak heights are such that a **distinct major contributor can be determined**. Any detected homozygous allele(s) of the major contributor is/are above the stochastic threshold. The detected alleles of the minor contributor may be above or below the PST.
        - For major/minor mixtures with seven or more alleles (including both major and minor components) at one or more loci, the minor component will be deemed as un-interpretable at this time and will not be used for comparison purposes.
        - If the "major" profile is neither clearly homozygous with all allelic peaks above the stochastic threshold nor has at least one allelic peak attributed to the major profile above the stochastic threshold if the locus is heterozygous, the technical reviewer must agree with the determination of "major/minor" for the DNA profile.

- If analysts wish to make a determination of "major/minor" but the profile has loci where both heterozygous alleles are below stochastic threshold, Technical Manager approval must be obtained.
- Determine which apparently homozygous peaks of the minor component are below the PST and therefore might not fully represent the minor component.
- If some loci show no minor component peaks, it is possible that either dropout of the minor component has occurred or the major component is masking the minor component. The profile as a whole must be considered as if the homozygous minor component alleles are below the PST. It must be considered that the true minor component may not be fully represented.
- If the minor component alleles are all above PST, the minor component can be considered fully represented. NOTE: In OSBI STATS, Possible Dropout will be marked "Yes" to account for the possibility for shared alleles with the major component.

- Option 2 – Mixture where the peak heights are such that a **distinct major contributor cannot be determined and all detected alleles are above the peak stochastic threshold**.
  - All alleles are detected and it is reasonable to assume no dropout due to all alleles being above the PST.
- Option 3 – Mixture where the peak heights are such that **a distinct major contributor cannot be determined and where some or all of the alleles are below the PST.**
  - Indistinguishable mixtures with peaks present below stochastic threshold will be treated as having a potential for dropout at all loci for statistical purposes.
- Option 4 –**Partial indistinguishable mixtures (including minor component mixtures of two or more individuals) must have interpretable results at a minimum of six loci for interpretation. Minor components determined to be single-source minor components must have interpretable results at a minimum of four loci for interpretation. Partial profiles that do not meet these requirements may be used for exclusionary purposes only if the technical reviewer agrees.** Profiles with results at more loci than these limits may also be deemed as inconclusive based upon complexity.

  When insufficient information is present in the questioned profile to make a determination regarding interpretation, these profiles will be deemed **not suitable for interpretation and/or comparison**. This determination could be due to the partiality of a profile or the extreme complexity of a profile.

5.2.5.4 **Comparisons of samples that appear to be from more than one source:**

5.2.5.4.1 If possible without causing uninterpretable results (such as off-scale data, excessive pull-up), analysts should attempt to re-amplify or re-extract in order to obtain a profile with all alleles above the peak stochastic threshold.

5.2.5.4.2 In general, when comparing a known reference sample to a questioned mixture profile, all allelic information from the known reference sample must be present in the mixture profile at all loci in order to report the comparison as an inclusion. However, the results of a comparison may occasionally be deemed an inclusion even though some alleles are not present in the mixture profile. In order to report an inclusion with some alleles not present in the mixture profile, the following criteria must be met:

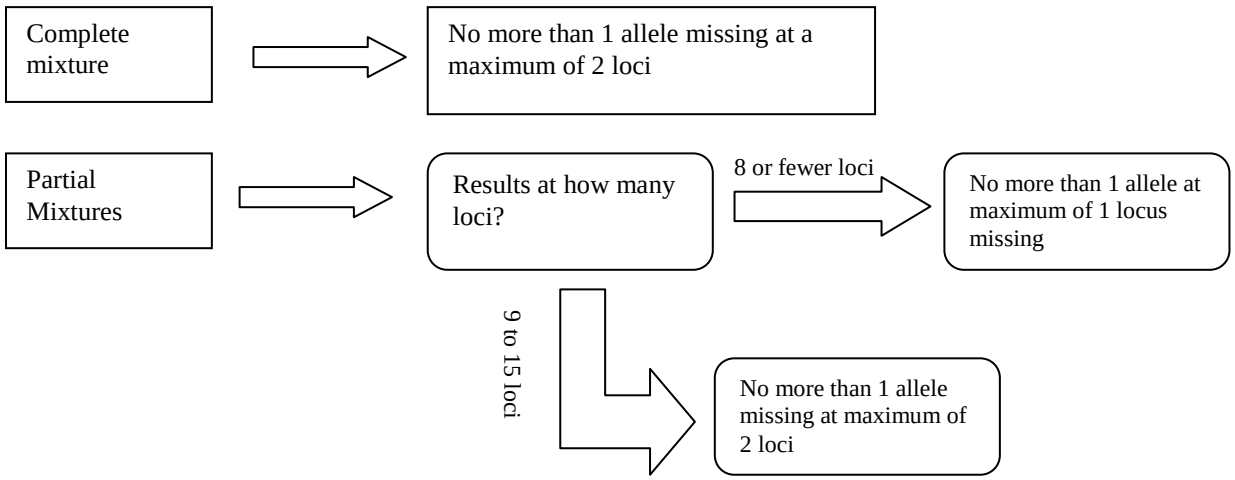
For a complete mixture profile, there must not be any more than one allele at a maximum of two loci where the alleles from the known reference sample are not present in the mixture.

For partial mixture profiles with no results at one or more locus/loci, there must not be any more than one allele at a maximum of one locus for a profile with results at eight or fewer loci (and a maximum of two loci for profiles with results at nine or more loci) where an allele from the known reference is not present in the mixture.

There must be a scientifically supported reason (degradation, inhibition) which explains the absence of the allele(s).

Comparison reasoning must be stated in the analyst's technical notes.

The Technical Manager must approve the inclusion for comparisons to partial mixture profiles if there is an allele from the known reference not present in the mixture profile.



5.2.5.4.3 Statistical calculations will be handled as referenced in CASE_6 below.

5.2.5.5 **Intimate Samples** - Evidence taken directly from an individual is expected to yield the DNA profile from this individual.

- Any mixture DNA typing results from such a sample that is consistent with the known individual, may be separated from the other mixture result to facilitate identification of foreign alleles (according to SWGDAM Guidelines) during data interpretation.
- A similar instance can arise when another known individual (consensual sexual partner) is also a contributor to the sample. The obligate alleles in these results can constitute a single-source or mixture profile, depending on the sample type.

5.2.5.6 If multiple results are obtained from an item or stain due to multiple extractions, amplifications, or genetic analyses, the most complete profile will be reported or it may be necessary to report multiple profiles.

5.2.5.7 The questioned samples will be analyzed with all peaks being appropriately designated and interpreted before comparisons of any known reference samples will be made. Sperm and non-sperm fractions from the same item can be used as an aid during the interpretation processes.

5.2.6 Types of Profiles

5.2.6.1 Single Source Profile Determination

- See above (CASE_5.2.5.1 and CASE_5.2.5.2).

5.2.6.2 Major/Minor Mixture Determination

- See above (CASE_5.2.5.3).

5.2.6.3 Indistinguishable Mixture Determination

- See above (CASE_5.2.5.3)

5.2.6.4 Partial Profile Determination:

- Degradation – if a sample is degraded, higher molecular weight alleles may not amplify due to preferential degradation of larger alleles.
- Locus (Allelic) Dropout – if an insufficient (or excess) quantity of human DNA template is used/available, there is a possibility for preferential amplification of certain loci/alleles over others. The result may generate some loci/alleles not amplifying at all. Inhibitors may also produce allelic dropout. This is usually seen in the higher molecular weight loci (CSF1PO, D2S1338, D18S51, and FGA).
    - Some partial profiles may be insufficient for comparison (CASE_5.2.5.2 and CASE_5.2.5.3 Option 4 states **"Partial indistinguishable mixtures**

(including minor component mixtures of two or more individuals) must have interpretable results at a minimum of six loci for interpretation.

Minor components determined to be single-source minor components must have interpretable results at a minimum of four loci for interpretation. Partial profiles that do not meet these requirements may be used for exclusionary purposes only if the technical reviewer agrees. Profiles with results at more loci than these limits may also be deemed as inconclusive based upon complexity.

When insufficient information is present in the questioned profile to make a determination regarding interpretation, these profiles will be deemed **not suitable for interpretation and/or comparison**. This determination could be due to the partiality of a profile or the extreme complexity of a profile".

5.2.6.5 Inconclusive Profile Determination:

- Some technical issues may prevent the complete interpretation of a DNA profile or a locus in a profile. Examples of such situations may include:
  - Accurate allele designations not possible due to a poor quality injection, the allelic ladder, or the sample itself.
  - Alleles which cannot be definitively assigned to a locus.
  - Controls for the sample did not perform properly.

CASE_6 DNA Statistical Analysis Guidelines (top ↑)

"Statistical interpretation for reported inclusionary results provides weight to the inclusionary statement." (SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories)

- **A statistical statement for all inclusionary, probative samples in order to report the weight of the match/non-exclusion will be included in the Criminalistics Examination Report.**

Statistical calculations reported that have drop-out indicated in OSBI STATS will include the following statement:

"The statistical calculation performed for this item accounts for potential missing genetic information due to low level/quality of DNA present and is a conservative estimate for the probability of finding someone unrelated that could also be a potential donor/contributor to the profile obtained."

Alternatively, this may be added to the statistics footnote as long as each item this applies to is listed in the statement.

- Statistical calculations reported where a locus has not been included for statistics in consultation with the Technical Manager will use the following wording:
  A DNA mixture of at least X people was obtained from Questioned Item Description (Item Q) with genetic information available at N of 16 genetic locations analyzed. Known Sample Name (Item K) cannot be excluded as a potential contributor at x of the locations. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to this mixture based on the same criteria that Known Sample Name (Item K) could not be excluded is at least 1 in X.

6.1 Single Source Autosomal STRs

- If a sample appears to be from a single source and two alleles are observed at a locus regardless of the PST or if a homozygous peak is observed above the PST, the analyst can be confident that the DNA profile at that locus is complete. These loci may be used for statistical calculations.

- If the sample appears to be from a single source, and only one allele is detected at a locus and the zygosity is in question (it is at or below the peak stochastic threshold), the analyst cannot be confident that the DNA profile is complete at that locus. In such instances, the analyst must indicate that the single peak is at or below the PST by making an indication of this observation on the profile table (i.e. bold/italicize this allele in the table). Depending on analyst discretion and interpretation (to be clearly articulated in the Technical Notes of the case file), for these profiles the following options exist:

  1) The formula $2p\text{-}p^2$ may be used to address this situation without double-counting the proportion of homozygotes in the population (according to *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories Section 5.2.1.3.3, approved January 2010*). This calculation will be performed using an in-house Excel spreadsheet (OSBI STATS) with allele frequencies from the FBI's Popstats Program and the STRbase website (for loci D2S1338 and D19S433). OSBI STATS was updated and performance checked in June 2015 (version 1.2) to include the amended allele frequencies provided by the FBI.

  2) The analyst may choose to perform no statistical calculations on such loci (according to *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories Section 5.2.1.3.3, approved January 2010*). Loci in this category may still be useful for exclusionary purposes in this circumstance.

- The random match probability of a given genetic profile shall be estimated using an in-house Excel spreadsheet (OSBI STATS) which utilizes the allele frequencies from the FBI's Popstats and STRbase website (for loci D2S1338 and D19S433).
- The probability reported in forensic autosomal casework by OSBI personnel shall be the probability generated by OSBI STATS (truncated using three significant figures), with the exception of criminal paternity cases which will use PopStats.

6.2 Major Components of Mixtures

- The major component or single source calculation shall be used for the major component of a mixture.

6.3 Minor Components of Mixtures

The minor component or indistinguishable mixture calculation shall be used for the minor component of a mixture based on the information available in the minor component.

- If any apparently homozygous allelic peaks are below the peak stochastic threshold (PST), the minor component may or may not be suitable for interpretation.

  Depending on analyst discretion and interpretation (to be clearly articulated in the Technical Notes of the case file), for these profiles the following options also exist for reporting this type of profile:

  1) The formula $2p - p^2$ may be used to address this situation without double-counting the proportion of homozygotes in the population (according to *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories Section 5.2.1.3.3, approved January 2010*). This calculation will be performed using an in-house Excel spreadsheet (OSBI STATS) with allele frequencies from the FBI's Popstats program and the STRbase website (for loci D2S1338 and D19S433).
  2) The analyst may choose to perform no statistical calculations on such loci (according to *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories Section 5.2.1.3.3, approved January 2010*). Loci in this category may still be useful for exclusionary purposes in this circumstance.

  If all loci have apparent homozygous peaks below the peak stochastic threshold in the minor component, the following wording may be used:

  The DNA profile from Questioned Sample Description (Item Q) does not satisfy the laboratory's inclusionary reporting criteria and therefore may be utilized for exclusionary purposes only. Based upon the results, the Known Sample Description (Item K) is excluded.

6.4 Indistinguishable Mixtures

To calculate the probability that a randomly selected person would be included as a contributor to the mixture, loci with alleles below the PST, the analyst will use the formula $2p - p^2$ to address this situation without double-counting the proportion of homozygotes in the population (according to *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories Section 5.2.1.3.3, approved January 2010*). This calculation will be performed using an in-house Excel spreadsheet (OSBI STATS) with allele frequencies from the FBI's Popstats program and the STRbase website (for loci D2S1338 and D19S433).

6.5 Partial Profiles

- Profiles that are partial shall be calculated using the appropriate formula (single source or mixture) at the loci where all alleles are deemed present above the peak stochastic threshold (PST) or the formula $2p - p^2$ may be used to address this situation without double-counting the proportion of homozygotes in the population for all loci in the questioned sample, since the statistic is reflective of the probability of selecting an unrelated individual at random from the population that would contribute this profile.
- If an inclusionary statement is reported for a partial profile, a statistical analysis must be performed. If the genetic information detected is too minimal, the profile will be deemed inconclusive or not suitable for interpretation at this time; however, once a profile is deemed "inconclusive", no comparisons can be made in the future to that profile without additional analyses of the sample being performed.

6.6 Y-STRs

- Calculations for Y-STR profiles are based on the counting method. The website (www.usystrdatabase.org) accepts the Y-STR allelic data and calculates the estimated frequency of the profile based on the data present in this database using the counting method.
- Statistics will be calculated for Partial or Full Single Source Y-STR Profiles and for the major component of Y-STR mixtures (that satisfy interpretation criteria in CASE_5).
- No statistical analysis shall be performed for Y-STR minor components ~~mixtures~~ or Y-STR indistinguishable mixtures (based on current SWGDAM guidelines).

6.7 Family Relatedness Statistics

- Likelihood Ratios (LR) will be used for family relatedness cases using the FBI's Popstats Program (when possible). If the calculations fall outside the capabilities of the Popstats Program, a hand calculation may be used. The appropriate formula will be used for each locus given the circumstances of the case (i.e. Known Mother with Alleged Father, Unidentified Remains with Alleged Parents). A combined likelihood ratio ($LR_{cumulative}$) will be determined by multiplying all the likelihood ratios calculated for each locus.
- $LR - H_0 / H_1$, where H_0 is the chance of producing a child genetically like this child from a

mating of the mother and alleged father and H_1 is the chance of producing a child genetically like this child from a mating of the mother with a randomly selected, unrelated man.

- In the case of a mutation, the following formula will be used:
  $LR = \mu/P_{ex}$, where μ is the mutation frequency and P_{ex} is the probability of exclusion.

- Once the LR is determined, the Posterior Probability will be determined with an assumption of 50% Prior Probability using the following formula:

$$Prob_{post} = [(0.5_{prior\ for})\ (LR)] / [(0.5_{prior\ for})\ (LR) + (0.5_{prior\ against})]$$

This probability will be reported using three significant figures (truncated).

ATTACHMENT(S):
SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories
SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing
OSBI STATS User Guide

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

CASE_7 Report Wording (top ↑)

7.1 Results must be reported for all evidence tested. In the event controls fail and tests cannot be repeated, report wording should indicate results are inconclusive due to control failure.

7.2 The following are recommendations for the wording of results of testing and are meant to be used as guidelines in reporting results. The wording may be altered or combined, as necessary, depending on case circumstances, analyst preference, or for clarification of results.

NOTE: Based on ASCLD/LAB-International requirements, when "sampling" is used, wording must be present in the report to indicate the results apply only to the portions of the item(s) tested.

7.3 Bloodstain Analysis—Hemastix and/or Takayama

If visual inspection showed no blood-like staining, so no chemical testing was performed, the following wording is recommended: "No blood-like staining was observed on. . ."

Use the following table to find the combination of tests performed and results obtained. Then look at the number indicated, and use that number to find the recommended report wording listed below.

RESULTS	Takayama Positive	Takayama Negative	Takayama Not Performed
Hemastix Positive	2	3	4
Hemastix Negative	2	1	1
Hemastix Not Performed	2	1	5,6

(1) "No blood was detected." or "Blood was not detected."
Analyst may also use wording such as: "(Number of) areas were tested on Item X for the presence of blood; however, no blood was detected." This then provides the number of areas/swabs/hairs tested on/from an item and what the results were. This type of report wording could be applied to all of the examples below.

(2) "Blood was detected."

(3) "Presumptive testing indicates the [possible] presence of blood; however, the [actual] presence of blood could not be confirmed."

(4) "Presumptive testing indicates the [possible] presence of blood; however, no confirmatory testing was performed."

(5) "No blood-like staining was observed."

(6) "Blood-like staining was observed, however, no further testing was performed."

7.4 Bloodstain Analysis—RSID

7.4.1 Positive RSID result: "Human blood was detected."

7.4.2 Negative RSID result: "Human blood was not detected."

7.4.2.1 Negative RSID result (with positive Takayama result): "Blood was detected; however, the blood is of non-human origin."

7.4.2.2 Negative RSID result (with positive Hemastix result): "Presumptive testing indicates the presence of blood; however, the [actual] presence of human blood could not be confirmed."

7.5 Sexual Assault Analysis

7.5.1 Crimescope (no further analysis)

7.5.1.1 "No staining consistent with seminal fluid was observed."

7.5.2 AP Spot Test

7.5.2.1 **Positive Result (with spermatozoa negative and p30 negative results)**

"Acid phosphatase activity was detected. All other tests for seminal fluid were negative."

7.5.2.2 **Negative Result**

"No acid phosphatase activity was detected."

7.5.3 Sexual Assault Analysis—p30 Detection Using Immunoassay and Microscopic Identification of Spermatozoa

RESULTS	Sperm Positive	Sperm Negative
P30 Positive	1	4
P30 Negative	3	5
P30 Not Performed	2	

(1) "p30 was detected/identified and sperm(atozoa) were observed/identified."
"Seminal fluid was detected/confirmed."

(2) "Seminal fluid was detected/confirmed."
"Sperm(atozoa) were observed/identified "

(3) "Seminal fluid was detected/confirmed ."

"Sperm(atozoa) were observed/identified."

"p30 was not detected/identified and sperm(atozoa) were identified."

(4) "p30 was detected/identified; however, no sperm(atozoa) were observed/identified."

"Seminal fluid was indicated "

(5) "No seminal fluid (or semen) detected/identified."

"p30 was not detected/identified and no sperm(atozoa) were identified."

NOTE: A report footnote may be included clarifying p30 such as "p30 is a protein found in high concentration in seminal fluid; however, this protein is also found in other body fluids at lesser concentrations. Therefore the detection of p30 is not confirmatory for the presence of seminal fluid but is only confirmatory for the presence of the protein p30."

7.6 Hair Analysis

7.6.1 Report wording should include the number of hairs selected for further DNA analysis.

7.6.2 Macroscopic Exclusion

"No apparent hairs were selected for nuclear (or mitochondrial) analysis."

"Apparent hairs were observed; however, no apparent hair(s) were selected for further analysis."

7.6.3 Microscopic Exclusion

"Non-human hairs were observed."

"Hairs of non-human origin were observed.

"(Human) Hairs have insufficient material present for both nuclear and mitochondrial DNA analysis."

7.6.4 Microscopic Examination (Nuclear DNA Analysis)

"(Human) Hairs were observed and potential cellular material appears to be present for nuclear DNA analysis."

"(Human) Hairs were observed and those hairs have an insufficient quantity of cellular material present for nuclear DNA analysis."

7.6.5 Macroscopic Examination (Mitochondrial DNA Analysis)

7.6.5.1 This must be reported only in cases where hairs are evaluated for potential mitochondrial DNA analysis.

"(Human) Hairs were observed and (are/are not) of optimum length for mitochondrial DNA analysis."

7.6.6 Macroscopic Hair Selection Wording

"Human hairs were observed on item_____and were macroscopically compared to a known hair sample from item____(Known from_____) in an attempt to determine the most suitable hairs for DNA analysis for this case. The hairs from item_____(have /do not have) similar characteristics to the known hair sample from_____. This/These hair(s) will (be/not be) forwarded for DNA analysis.

7.6.7 Inconclusive Results

If the analyst is unable to determine whether the hair is human or non-human:

"Hairs were observed but a determination of human/non-human origin could not be made based on the characteristics observed."

7.6.8 Debris and/or Possible Fiber Collection

"During the examination of item(s)_____, debris and possible fiber(s) were observed that may be suitable for additional testing. No further analysis was performed at this time."

"Debris/Possible fiber(s) was/were collected from item(s)_____but was/were not analyzed at this time."

7.7 DNA Analysis

7.7.1 Results and/or Conclusions for all samples where DNA analysis was attempted must be reported. Samples taken from the same parent stain/swab may be reported as one profile from the analysis that provides the most genetic information.

7.7.2 All known reference samples shall be compared to all forensic samples.

7.7.3 Each DNA report will contain the following elements:

7.7.3.1 The method of analysis and the loci amplified.

"Deoxyribonucleic Acid (DNA) was isolated from the following item(s) (or list items) and characterized through the Polymerase Chain Reaction (PCR) using the Short Tandem Repeat (STR) Identifiler® Plus amplification kit (and/or male-specific Short Tandem Repeat (Y-STR) Yfiler™ amplification kit)."

For differential extractions, a statement will be made to inform the customer.

"The analysis of Item X included separating the item into two fractions/portions: an epithelial fraction/portion and a sperm fraction/portion."

7.7.3.2 For any references to profiles generated outside of the OSBI, the laboratory conducting the analysis will be identified.

7.7.3.3 The type of profile identified.

- No DNA profile was obtained.
- A full, single source profile was obtained.
- A partial DNA profile was obtained (single source or mixture).
- The report wording must reflect that a partial profile was obtained either by using the wording "partial profile" in the results statement or listing the number of loci at which the profile has been identified if fewer that the number of loci amplified.
- A mixture of DNA was obtained that was able to be separated into a major component and a minor component.
- An indistinguishable mixture DNA profile was obtained.

7.7.3.4 A statement of inclusion, exclusion, or inconclusive.

Inclusionary Statements – The reporting of a positive association between a questioned profile and a reference profile. There are two types of inclusionary statements:

- Match Statement – applies to single source profiles or profiles deduced to single genotypes at all loci and should indicate to whom the questioned profile matches.
- Non-Exclusion Statement – applies to mixtures where profiles cannot be deduced to single genotypes at all loci (i.e. major components) and should indicate who cannot be excluded as a possible contributor. This statement also applies to single source profiles which exhibit possible incomplete genotypes at any locus.

Exclusionary Statements – The reporting that no positive association exists between a questioned profile and a reference profile. There are two types of exclusion statements:

- Does Not Match Statement – applies to single source profiles or profiles deduced to single genotypes at all loci (i.e. major components) and should indicate to whom the profile does not match.
- Is Excluded Statement – applies to mixtures where profiles cannot be deduced to single genotypes at all interpretable loci and should indicate who is excluded as a possible contributor. This statement also applies to single source profiles which exhibit possible incomplete genotypes at any locus.

Not Suitable for Interpretation / Inconclusive Statements – Pertains to comparisons where there is insufficient information in a questioned profile to exclude an individual, but also insufficient information to conduct a statistical evaluation. In such instances, the following statement will be used:

Based on the genetic information available and the possibility of other genetic information being present that may be undetected by this testing procedure, no conclusions can be made with regards to the comparison of Known Sample Name (Item K) to Item Q. Known Sample Name (Item K) can neither be included nor excluded as a contributor to Item Q.

7.7.3.5 Any additional applicable information about the evidence.

7.7.3.6 Any request to the submitting or requesting agency regarding additional evidence or information needed.

7.7.4 **Inclusion, Exclusion, and Not Suitable / Inconclusive Statements -** The examples given of inclusion, exclusion, no interpretive conclusions, and inconclusive report wording are categorized under the situation which most commonly warrants them. Although the intent of providing the report wording in this section is to standardize report wording, the conclusion statements below are intended as a general guideline and can be modified as needed based on case circumstances. These guidelines are not an exhaustive list of all scenarios. Final discretion regarding interpretation is left to the analyst, in consultation with the Technical Manager if needed.

7.7.5 All DNA inclusions must contain a statistical statement, excluding intimate samples. Statistics are not required for exclusionary conclusions, inconclusive/un-interpretable results or comparisons between multiple questioned samples with no comparison to a known reference sample. The OSBI Forensic Biology Units shall report out the most conservative statistic from the three most common ethnic/population groups in Oklahoma: Caucasian, African American, and Southwest Hispanics. The statistical information shall be added as the final statement of the inclusion wording. The number reported shall be no more than three significant figures (truncated).

7.7.5.1 The statistical statement wording has been included in the following statements for clarity; however, this wording is not necessary for exclusionary statements.

7.7.5.2 If statistics are not reported on an intimate sample, the following statement must be added to the report wording for that item:

Due to the intimate nature of the sample, Known Sample Name (Item K) is presumed to be the source of this DNA profile.

7.7.6 **Results/Conclusions for Forensic Single Source Samples**

Single Source (Full Profile):

- *The DNA profile [from the Questioned Sample Description (Item Q)]* ***matches*** *the DNA profile from Known Sample Name (Item K). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*

- *The DNA profile [from Questioned Item Description (Item Q)]* ***does not match*** *Known Sample Name (Item K); he/she is* ***excluded*** *as a potential donor.*

Partial Single-Source Profile:

Partial single source profiles must have a result at a minimum of four loci for interpretation. A partial single source DNA profile where all alleles are accounted for by the known reference sample, but up to one allele per locus present in the known standard do/does not appear in the questioned sample for scientifically supported reasons:

When all the alleles from the known reference sample are represented in the questioned sample at all loci where results are obtained:

- *A partial DNA profile was obtained [from Questioned Item Description (Item Q)] [with genetic information available at X of 16 locations analyzed]. Known Sample Name (Item K)* ***cannot be excluded*** *as a potential donor. The probability of selecting an unrelated individual at random from the population having this partial DNA profile is at least 1 in X.*
- *The DNA profile from Known Sample Name (Item K)* ***is consistent*** *with the partial DNA profile obtained [at X of 16 loci] from Questioned Item Description (Item Q).*

The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.

- *Known Sample Name (Item K) is **excluded** as a potential donor.*

When there are alleles from the known reference sample not present in the questioned sample at loci where results were obtained:

- *Known Sample Name (Item K) **cannot be excluded** as a possible source of the partial DNA profile obtained (at number of loci) from Questioned Item Description (Item Q). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*

Partial Profile Insufficient for Comparison:

- *A partial DNA profile with very limited genetic information was obtained [from Questioned Item Description (Item Q)]. It is not suitable for interpretation and no conclusions can be made at this time.*

If many of the alleles are missing where an analyst is not confident in the match or both alleles in a locus from the known are missing from the questioned sample:

- *No conclusions can be made regarding comparisons of Questioned Sample Description (Item Q) to Known Sample Name (Item K) due to genetic information being undetected, and the possibility of other genetic information being present that may be undetected by this testing procedure. This profile does not contain enough genetic information to either exclude or imply any positive association with Known Sample Name (Item K).*

Full Single Source But Known Exhibits Partial Profile

If the known sample exhibits a partial profile (degraded reference sample) (at number of loci), the wording "is consistent with" will be used instead of "match" when any inclusionary statements are made regarding a questioned sample and the partial known sample. Statistics for the comparison will include only those alleles in the questioned sample at the loci corresponding to those that have results in the known profile in order to not overstate the strength of this inclusion since the statistic is provided in order to give a weight to the match or inclusion.

- *The DNA profile obtained **is consistent** with the partial DNA profile obtained from Known Sample Name (Item K) (at X genetic locations yielding results). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*
- *The DNA profile **does not match** (the partial DNA profile obtained from) Known Sample Name (Item K); he/she is **excluded** as a potential donor.*

7.7.7 Results/Conclusions for Forensic Multi-Person Mixtures:

Differential Extractions – Reporting each Fraction

Each fraction from differential extractions will be reported as indicated below, depending on the results obtained from the fraction.

Major/Minor Mixture (no dropout, single source minor):

The DNA profile obtained [from Questioned Sample Description (Item Q)] is a mixture that can be separated into a major and minor component.

The major component ***matches*** *the DNA profile obtained for Known Sample Name (Item K). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*

The DNA profile from the major component ***does not match*** *Known Sample Name (Item K); he/she is excluded as a potential donor.*

Known Sample Name (Item K) ***cannot be excluded*** *as a potential contributor to the minor component. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to the minor component is at least 1 in X.*

Known Same Name (Item K) is ***excluded*** *as a potential contributor to the minor component.*

Major/Minor Mixture (dropout indicated):

The DNA profile obtained [from Questioned Sample Description (Item Q)] is a mixture that can be separated into a major and minor component.

The DNA profile from the major component ***matches*** *Known Sample Name (Item K). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*
The DNA profile from the major component ***does not match*** *Known Sample Name (Item K); he/she is* ***excluded*** *as a potential donor.*

Known Sample Name (Item K) ***cannot be excluded*** *as a potential contributor to the minor component. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to the minor component is at least 1 in X.*
Known Sample Name (Item K) is ***excluded*** *as a potential contributor to the minor component.*

Major/Minor Mixture (Minor >1 person / Indistinguishable Mixture:

The DNA profile obtained [from Questioned Sample Description (Item Q)] is a mixture that can be separated into a major and minor component.

The DNA profile from the major component ***matches*** *Known Sample Name (Item K). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*
The DNA profile from the major component ***does not match*** *Known Sample Name (Item K); he/she is* ***excluded*** *as a potential donor.*

The minor component appears to be a (partial) mixture (of at least X individuals). Known Sample Name (Item K) cannot be excluded as a potential contributor. The probability of selecting an unrelated individual at random from the population who could be a contributor to this minor component is at least 1 in X.
Known Sample Name (Item K) is ***excluded*** *as a potential contributor to the minor component.*

Major/Minor Mixture (Minor Insufficient for Comparison):

The DNA profile obtained [from Questioned Sample Description (Item Q)] is a mixture that can be separated into a major and minor component.

The DNA profile from the major component ***matches*** *Known Sample Name (Item K). The probability of selecting an unrelated individual at random from the population having this DNA profile is at least 1 in X.*
The DNA profile from the major component ***does not match*** *Known Sample Name (Item K); he/she is* ***excluded*** *as a potential donor.*

The minor component of this mixture contains limited genetic information and is insufficient for comparison.

Indistinguishable Mixtures

Indistinguishable Mixture (No Dropout Indicated):

A DNA mixture [of at least X individuals] was obtained [from Questioned Sample Description (Item Q)]. Known Sample Name (Item K) ***cannot be excluded*** *as a potential contributor. The probability of selecting an unrelated individual at random from the population that could be a contributor to this mixture is at least 1 in X.*
Known Sample Name (Item K) is ***excluded*** *as a potential contributor.*

Indistinguishable Mixture (Dropout Indicated):

A partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (Item Q)]. Known Sample Name (Item K) ***cannot be excluded*** *as a contributor. The probability of selecting an unrelated individual at random from the population who could be a contributor to this mixture is at least 1 in X.*

Known Sample Name (Item K) is ***excluded*** *as a contributor to this mixture.*

Partial Indistinguishable Mixture (Dropout Indicated):

A partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (Item Q)] with genetic information available at X of 16 locations analyzed. Known Sample Name (Item K) ***cannot be excluded*** *as a contributor. The probability of selecting an unrelated individual at random from the population who could be a contributor to this mixture is at least 1 in X.*

Known Sample Name (Item K) is ***excluded*** *as a contributor to this mixture.*

Indistinguishable Mixtures with 7 or More Alleles at a Locus:

If a profile is not interpreted due to the complexity (greater than 7 alleles at a locus):

> *A DNA mixture was obtained [from Questioned Item Description (Item Q)]. Due to the complexity of the mixture obtained, it is not suitable for interpretations and no conclusions can be made at this time. (Optional additional wording: Known Sample Name (Item K) can neither be included nor excluded as a contributor to Item Q).*

NOTE: While every effort should be made to reliably draw conclusions from mixture samples with no more than seven alleles at a locus, some mixtures may not lend themselves to interpretation using the above outlined procedures. Alternatively, such mixtures may yield typing information only for exclusionary purposes only. In these instances, report wording may be as follows:

> *The DNA profile from Questioned Sample Description (Item Q) indicates a mixture. The DNA profile obtained from Item Q does not satisfy the laboratory's inclusionary reporting criteria and therefore may be used for exclusionary purposes. Based on the results, the Known Sample Name (Item K) is excluded as a potential contributor to the mixture.*

Inconclusive Partial Mixture:

An inconclusive partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (Item Q)] with genetic information at only X of 16 locations analyzed [list loci if desired]. There is not enough genetic information to either exclude or imply any positive association with Known Sample Name (Item K).

Relatedness (Father, Mother, and Child/Children):

A DNA mixture (of at least X individuals) was obtained. Known Sample Name (Item K) ***cannot be excluded*** *as a potential contributor. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to this mixture is at least 1 in X. However, given that Biological Father Sample Name (Item F) and Biological Mother Sample Name (Item M) are the biological parents of Child/Children Known Sample Name(s) (Item C) and the subsequent family relatedness between these individuals (i.e. sharing of genetic information known to occur between family members), no further interpretive conclusions can be made regarding the source of the Questioned Description Item (Item Q) at this time.*

7.7.8 Results/Conclusions for Profiles with No Known Samples

A (partial/full) (profile/mixture) was developed from Questioned Sample Description (Item Q) [at number of loci]. Please submit known samples for comparison purposes.

7.7.9 Results/Conclusions for Paternity Cases

Inclusion:

Alleged Father Name (Item AF) cannot be excluded with a ($Prob_{post}$) probability as the biological father of the Child Name (Item C), given Mother Name (Item M) is the biological mother of the child.

Exclusion:

Alleged Father Name (Item AF) is excluded as a biological parent of the Child Name (Item C), given Mother Name (Item M) is the biological mother of the child.

NOTE: If all paternal obligate alleles are not present, but could be accounted for by mutation, etc., recommend that the Agency contact an appropriate outside parentage laboratory for further analyses.

7.7.10 Results/Conclusions for Profiles Matching Staff

Single Source: *An identified profile (profile consistent with OSBI personnel) was obtained from Questioned Sample Description (Item Q) and the data were determined to be unreportable.*

Mixture: *DNA from more than one individual was obtained from Questioned Sample Description (Item Q). An identified profile was obtained in this sample and the data were determined to be unreportable. No further conclusions can be made regarding the DNA profile from this item.*

7.7.11 Results/Conclusions for Samples with Associated Controls that were Compromised

The results obtained from the Questioned Sample Description (Item Q) could not be interpreted due to technical limitations. An insufficient quantity of the item is available for retesting (if applicable).

7.7.12 Results/Conclusions for Insufficient Profile (doesn't meet OSBI reporting requirements)

The DNA profile obtained from Questioned Sample Description (Item Q) was insufficient for comparison.

7.7.13 Results/Conclusions for No DNA Profile Obtained

No DNA profile was obtained from Questioned Sample Description (Item Q).

7.7.14 Results/Conclusions for Extracted DNA Not Amplified

DNA was isolated from Questioned /Known Sample Description (Item Q/K). No further analysis was performed at this time.

Inconclusive (i.e. technical issues, allele resolution issues, associated controls affected, deemed not suitable for interpretation / inconclusive by analyst during interpretation even though it meets reporting requirements – reason articulated in case record)

- *An inconclusive DNA profile (or profile not suitable for interpretation) was obtained from Questioned Sample Description (Item Q) (at the A, B, C, and D loci).*

Miscellaneous Statements

Questioned Sample Description (Item Q) was not profiled [at this time].

Questioned Sample Description (Item Q) was not analyzed [at this time].

Evidence Matching Staff:

If the controls were compromised and the testing of the Item cannot be reported:

The results obtained from Questioned Sample Description (Item Q) could not be interpreted due to technical limitations.

For DNA mixtures where all of the alleles in the mixture are not accounted for by the known reference samples provided:

The DNA profiles of Known Sample Name(s) (Item(s) K) do not account for all of the genetic information obtained from this DNA mixture.

7.7.15 Results/Conclusions for Y-STR Analysis

7.7.15.1 Partial and Full Single Source Profiles

The (partial) Y-STR DNA profile obtained from Questioned Sample Description (Item Q) ***matches/is consistent*** *with the Y-STR DNA profile obtained from Known Sample Name*

(Item K). Therefore, Known Sample Name (Item K) (and all his paternal male relatives for inclusions) are ***included*** *as a potential donor (or source) of the Questioned Sample Description (Item Q). Using a published Y-STR DNA population database, this Y-STR profile has been observed in X of X total individuals within the United States Y-STR Database.*

The (partial) Y-STR DNA profile obtained from Questioned Sample Description (Item Q) ***does not match*** *with the Y-STR DNA profile obtained from Known Sample Name (Item K). Therefore, Known Sample Name (Item K) (and all his paternal male relatives for inclusions) are* ***excluded*** *as a potential donor (or source) of the Questioned Sample Description (Item Q).*

7.7.15.2 Y-STR Major Component Mixture Profiles

The (partial) Y-STR DNA profile from Questioned Sample Description (Item Q) appears to be a mixture. This mixture can be separated into a major component and a minor component.

The (partial) Y-STR DNA profile obtained from the major component of Questioned Sample Description (Item Q) ***matches/is consistent with*** *the Y-STR DNA profile obtained from Known Sample Name (Item K). Therefore, Known Sample Name (Item K) (and all his paternal male relatives for inclusions) are included as a potential donor (or source) of the major component of the Questioned Sample Description (Item Q). Using a published Y-STR DNA population database, this Y-STR profile has been observed in X of X total individuals within the United States Y-STR Database.*

The (partial) Y-STR DNA profile obtained from the major component of Questioned Sample Description (Item Q) ***does not match*** *the Y-STR DNA profile obtained from Known Sample Name (Item K). Therefore, Known Sample Name (Item K) (and all his paternal male relatives for inclusions) are* ***excluded*** *as a potential donor (or source) of the major component of the Questioned Sample Description (Item Q).*

7.7.15.3 Y-STR Indistinguishable Mixtures and Minor Component Profiles

At this time no inclusions will be made for Y-STR minor components or Y-STR indistinguishable mixtures. If a Y-STR minor component or Y-STR indistinguishable mixture profile is obtained, and a known profile cannot be excluded the following language should be used:

A(n) (indistinguishable mixture/minor component) Y-STR profile was obtained from Questioned Sample Description (Item Q).

No interpretive conclusions for (this sample/ the minor component of this sample) were made at this time.

The Y-STR DNA profile from Questioned Sample Description (Item Q) appears to be a mixture. The source of Known Sample Name (Item K) is ***excluded*** *as a potential contributor to this mixture.*

7.7.15.4 Partial or Incomplete Results for Y-STR profiles:

The partial Y-STR DNA profile obtained from item is insufficient for comparison. Due to the partial nature of the Y-STR DNA profile obtained from Questioned Sample Description (Item Q), Known Sample Name (Item K) can be neither included nor excluded from the mixture Y-STR profile obtained from Questioned Sample Description (Item Q).

7.7.16 Disposition Statement

All evidence (list specific Item(s)) will be retained at the OSBI Forensic Science Center/Northeast Regional Laboratory.

All evidence (list Items) will be returned to the requesting agency and should be retained.

7.7.17 CODIS Statement

The following (or similar) wording will be added to each DNA report indicating whether any/which profiles were forwarded for entry into the CODIS database:

<u>For cases where there are profiles identified that are eligible for entry:</u>

(The) DNA profile(s) from items (list item numbers) have been entered into the CODIS database.

<u>For cases where no profiles are identified that are eligible for entry:</u>

No DNA profiles were identified which were eligible for entry into the CODIS database.

7.7.18 Report Footnotes

- Each report will contain a footnote indicating:

  This report contains the conclusions, opinions, and interpretations of the analyst whose signature appears on the report.

If statistics are used in a report, the following footnotes will also be included, as applicable:

- ***Y-STR***:

  Statistics calculated using US Y-STR database. (http://www.usystrdatabse.org and version, if available).

- ***STR (non-parentage)***:

  Statistics were calculated using the OSBI statistics program: "OSBI STATS, Version X," which calculates statistics for the population groups: Caucasian, African American, and

Southwest Hispanic using allele frequencies from the Federal Bureau of Investigation (FBI) 2015 amended Popstats program and the Short Tandem Repeat DNA Internet Database (STRbase).

Statistical calculations reported that have drop-out indicated in OSBI STATS will include the following statement:

The statistical calculation performed for this item accounts for potential missing genetic information due to low level/quality of DNA present and is a conservative estimate for the probability of finding an unrelated individual at random from the population that could also be a potential donor/contributor to the profile obtained.

- ***STR (parentage)***:
  Statistics were calculated using the Federal Bureau of Investigation (FBI) Popstats program. This program calculates statistics for the Caucasian, African American, and Southwest Hispanic population groups.

ATTACHMENT(S):
SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories
SWGDAM Interpretation Guidelines for Y-Chromosome STR Testing
Guide for Determining What is Allowable in the Forensic Index at NDIS

REFERENCE(S):
OSBI CSD Quality Manual
ISO/IEC 17025:2005
ASCLD/LAB-*International* Supplemental Requirements – Testing
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

CASE_8 Retention of Samples (top ↑)

8.1 Serology Samples

BLOOD TESTING work products

Hemastix	Not Applicable
Takayama	Slides generated while performing the Takayama test are to be disposed of after analysis of the sample is completed.
Human Blood Testing	Cuttings generated while performing the RSID-Blood test are to be disposed of after analysis of the sample is completed.

SEMINAL FLUID TESTING work products

AP Spot Test	Cuttings generated while performing the AP Spot test are to be disposed of after analysis of the sample is completed.
p30 Detection	Cuttings generated while performing the p30 test are to be disposed of after analysis of the sample is completed.
Microscopic ID of Sperm	Cuttings generated while performing the sperm cell search test are to be disposed of after analysis is complete. The slide prepared from these samples will be handled as outlined below: POSITIVE RESULT: Slides with a positive result may either be retained with other retained items of evidence in the case at the respective OSBI Laboratory or may be returned to the requesting agency with an indication that the agency is required to retain this/these positive biological sample(s) in accordance with Title 22 § 1372 (i.e. by placing a sticker with this indication on the outer packaging). NEGATIVE RESULT: Slides with a negative result may be disposed of after the review of the case is complete or they may be returned to the requesting agency with the other negative items in the case.

8.2 DNA Samples

- Samples will be handled according to QP 6.1 – Evidence Handling, QP 6.2 – Evidence Handling for Non-Casework Purposes, QP 6.3 – Evidence Storage and Maintenance, QP 6.4 – Evidence Refrigerator and Freezer Maintenance.
- Cuttings, extracted DNA, and corresponding controls, at a minimum, shall be retained by the

OSBI if the sample was consumed and extract remains, or additional analysis may be necessary (for example, Y-STR analysis). In addition, if case circumstances warrant, the cuttings, extracts, and controls may be retained even if they do not fall under the above conditions. These retained samples should be stored frozen in a heat-sealed, clear plastic bag.

- Amplified DNA products and Genetic Analyzer samples shall not be retained. After the technical review has been performed and the review form signed, all amplified DNA and genetic analyzer plates must be disposed of in a biohazard container.

8.3 Documentation Requirements

- The case notes should clearly describe what evidence was retained.
- The case notes should clearly describe what cuttings, extracts, and dilutions were prepared and whether the samples were retained.

REFERENCE(S):
OSBI CSD Quality Manual
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

FBU Quality Control Procedures

QC_1 CLEANING AND DECONTAMINATION (top ↑)

1.1 General Decontamination

1.1.1 Instruments

- Instruments used to process biological samples (forceps, scissors, etc.) shall be cleaned with an approximate 1:10 bleach solution or alcohol before and after each sample is handled.
- Exposure to ultraviolet light for a minimum of 15 minutes in a UV crosslinker should be used to decontaminate articles which are small enough to fit into the unit and that will not be damaged by the ultraviolet light

1.1.2 Equipment

- Equipment shall be decontaminated with an approximate 1:10 bleach solution periodically.
- This shall be documented on the Decontamination Log (QC_DecontamLog) associated with the equipment or instrument.

1.1.3 Floors

- Use an approximate 1:10 bleach solution for decontamination of floors (as necessary).

1.1.4 Work Surfaces

- Each separate biological sample shall be handled on a clean sheet of paper or benchkote-like material to minimize the potential for work surface contamination.
- Work surfaces shall be cleaned with an approximate 1:10 bleach solution both before **and** after use.

1.1.5 General Laboratory Glassware and Plasticware

- Glassware and plastic containers can be cleaned with detergent and completely rinsed with tap water, and then distilled water by hand immediately after use.
- An automatic dishwasher may be used instead of hand washing. If a dishwasher is used, glassware must be rinsed with distilled water either after washing or before use.

1.1.6 Glassware and Plasticware Used to Handle Biohazards

- Clean and sterilize any item used to handle biological samples following such a procedure.
- Rinse item with approximate 1:10 bleach solution or a cleaner labeled as tuberculocidal and then wash as above with detergent.
- In addition to above, ultraviolet light exposure for ~15-30 minutes can be used for decontamination.
- In addition to above, steam and/or heat sterilization in an autoclave can be used for items that

will not melt.

1.1.7 Solutions, utensils, and tubes necessary for DNA analysis

- Should be sterilized by exposure to UV light for a minimum of 15 minutes in a UV crosslinker prior to use.

1.2 Solutions and Supplies Requiring Sterilization

1.2.1 Autoclaving

- Autoclaving of solutions, if required, should be performed at the time of preparation.
- Steam autoclaving of solutions should be performed at ~120°C for ~20 minutes following the instructions from the autoclave operations manual.
- Use autoclave indicator tape on the containers.
- After autoclaving is complete, check the indicator tape to make sure sterilization has taken place. If tape has not turned black, repeat procedure.

1.2.2 Ultraviolet (UV) Crosslinker

- If crosslinking is required, items will be exposed to UV light (short wave: 254 nm) following instructions within the crosslinker operations manual for minimum of 15 minutes.

1.3 Decontamination Following a Contamination Event

Following any contamination event, the OSBI Forensic Biology Units should perform a standardized decontamination procedure after the remediation method has been determined. The following may be utilized.

- Discard facemask and obtain a new facemask.
- Remove laboratory coat from the laboratory and have it cleaned or obtain a new disposable lab coat.
- Dispose of aliquots of reagents such as TE^{-4}, extraction reagents, and any kit reagents that have been tested and shown to be contaminated.
- Decontaminate (with 1:10 bleach) all supplies (pipettors, racks, de-cappers, etc.) and equipment (centrifuges, hoods, etc.) used throughout analysis process.
- Decontaminate (with 1:10 bleach) all work areas including common areas potentially used throughout the analysis process.
- UV crosslink all small supplies and reagents associated with the analysis process.

ATTACHMENT(S):
FBU Policy Manual QC_DecontamLog

FBU Quality Control Procedures

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_2 pH Meter (top ↑)

2.1 Individual Steps of the Procedure

2.1.1 Monthly Maintenance Requirements

- Wipe any salt deposits off electrode.
- Add filling solution to electrode, if necessary.
- Document maintenance on pH Meter Maintenance and Calibration Log (QC_pHMeter).

2.1.2 As Needed Maintenance

Clean electrode. Recommended Cleaning Procedures include one of the following:

- soak electrode in 0.1 M HC1 or 0.1 M Nitric Acid for one-half hour
- soak electrode in 1:10 dilution of household laundry bleach or in a 0.1-0.5% liquid detergent solution in hot water with vigorous stirring for 15 minutes
- procedure detailed in the operator's manual for that electrode
- Change electrode storage solution

After cleaning, drain and refill the reference chamber and soak the electrode in storage solution for at least 1 hour.

2.1.3 Calibration

Calibrate the pH meter on the day of use prior to testing samples.

- Select two (2) standardized reference buffer solutions that bracket the expected pH of the sample to be tested.
- Follow the manufacturer's instructions as listed in the operator's manual.
- If the slope falls within the specifications as listed in the operation's manual, measurement of samples may proceed. Slope may not be available on all pH meters; therefore, this measurement may not be applicable.

Document calibration on pH Meter Maintenance and Calibration Log (QC_pHMeter).

2.1.4 Measurement

- Rinse electrode with distilled water and wipe away excess with a tissue.
- Place the electrode in the sample. Gently swirl sample if needed.
- When display is stable, record pH.

2.2 pH Meter Performance

- The slope must fit within the recommended range as indicated by the user's manual.
- If the slope falls outside the range specified by the manual, make sure the discrepancy is not due to a

problem with the electrode.

- If the problem is not due to the electrode but due to the pH meter itself, it should be taken out of service and the manufacturer contacted for repair.
- If the problem is due to the electrode, the electrode should be replaced, and the faulty electrode properly disposed of.

ATTACHMENT(S):
FBU Policy Manual QC_pHMeter

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_3 Water Purification Systems (top ↑)

3.1 Individual Steps of the Procedure

3.1.1 Maintenance—Final Filter Replacement
The final filter must be replaced when the SynergyPak is replaced or if the product water flow rate drops.

3.1.2 Maintenance—Flow Calibration
Flow calibration must be performed when the final filter is replaced or as needed.

3.1.3 Maintenance—SynergyPak Replacement
The SynergyPak should be replaced when the Pack Alarm display or System Resistivity Display is blinking.

3.1.4 Maintenance—UV Lamp Replacement and UV Timer Reset
UV Lamp should be replaced and the UV Timer should be reset when the UV Lamp Alarm is blinking.

3.1.5 Maintenance—Screen Filter Cleaning
Screen filter cleaning should be performed two (2) times a year or as necessary.

3.1.6 Document maintenance on the Water Purification System Maintenance Log (QC_H2O).

3.2 Water Purification System Performance

- The expected resistivity should read 18.2 MΩ cm @ 25°C.
- If this reading is not between 10 and 18.2, recirculate the water in the system
- If this does not alleviate the problem, then the SynergyPak should be replaced.

ATTACHMENT(S):
FBU Policy Manual QC_H2O

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_4 Refrigerators and Freezers (top ↑)

4.1 Individual Steps of the Procedure

- At least once a week, temperatures shall be recorded on the Temperature Log (QC_Temp). The analyst recording the temperature shall check the records for any variances from the expected value that may show the performance of the unit over several measurements is not satisfactory.
- The monitoring probes should be placed such that they are not in an area where the temperature fluctuates enough to set off the alarm during defrost cycles.
- The alarm monitoring pad should be set to take the variance into account.
- The weekly temperature should not be recorded when the unit is in defrost mode.
- Equipment can be inspected at this time for mold, mildew, excess frost/ice buildup or any other possible deleterious condition that may require maintenance. Any maintenance shall be recorded on the equipment maintenance log.

4.2 Unsatisfactory Refrigerator or Freezer Performance

- The unit shall be inspected and the cause of any variance determined and corrected. If the cause cannot easily be determined or corrected by in-house personnel, the unit shall be emptied and placed "out of service" until function is restored.
- The contents shall be transferred to a working unit (if possible) or to a temporary storage container until expedient arrangement can be made for proper storage.
- If a unit sets off an alarm, the alarm monitoring company, as per contract, should contact, in a timely manner, the designated contact person for that laboratory. The contact person shall notify, if necessary, the appropriate unit supervisor or his/her designee in a timely manner. The supervisor/designee shall take the action described above.

FBU Quality Control Procedures

ATTACHMENT(S):
FBU Policy Manual QC_Temp

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories
OSBI CSD Quality Manual QP 8.1 – Storage of Reagents and Chemicals

QC_5 Centrifuges (top ↑)

5.1 Cleaning

- Use neutral cleaning agents to clean the centrifuge housing, rotor chamber, and rotor accessories.
- Ensure that all parts are dried thoroughly by hand before use.

5.2 Performance Check - At a minimum, the OSBI Forensic Biology Units shall perform an annual performance check for each centrifuge consisting of both a RPM calibration and Timer calibration.

If the performance check is not completed within the anniversary month of the last check, the centrifuge shall be removed from service until a performance check is successfully completed. Performance checks are required for only those centrifuges used where the RPM and time measurements are required in case work; performance checks are not required for centrifuges designated as "QUICK SPIN ONLY".

5.2.1 RPM Calibration

- The speed (RPM) as measured by a tachometer using a strobe light.
- Ensure that there is a reflective surface on the rotor of the centrifuge to be checked which shall register when the tachometer is activated.
- Turn on the centrifuge and allow the centrifuge to come to maximum rotation.
- Follow the operations manual for the tachometer to begin the calibration of the centrifuge.
- Document RPM calibration results on the Centrifuge Calibration Log (QC_Cent).

5.2.2 Timer Calibration

- Measure the timer against a stopwatch or other calibrated timer set for the same time interval(s).
- Document timer calibration results on the Centrifuge Calibration Log (QC_Cent).

5.3 Centrifuge Performance

5.3.1 RPM

- RPM must fall within the desired range (10,000 rpm $\leq$ x $\leq$ 15,000 rpm for microcentrifuges).
- Moderate changes in RPM may be an indication that preventive maintenance or repair is necessary
- If the centrifuge RPM falls outside of the minimum and maximum as set by this protocol, the microcentrifuge should be taken out of service and the authorized service representative for that model should be contacted for service.

5.3.2 Timer

- If the timer varies more than 30 seconds in five minutes, the variation should be documented and a correction factor should be placed with the centrifuge or an external timer used.

ATTACHMENT(S):
FBU Policy Manual QC_Cent

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_6 NIST Certified Equipment (top ↑)

6.1 Individual Steps of the Procedure

- The calibration for weights, timers, tachometers, and delegated temperature verification systems is to be performed annually or prior to the expiration date of the current NIST certificate, whichever is longer. Thermometers must be calibrated annually.
- Contact a company/business that is an authorized NIST certifying body, such as Chermac Services Corporation at (405) 340-9326, or the NIST Calibration Program at (301) 975- 2092 or calibrations@nist.gov to make arrangements for the calibration.
- Once IPR is approved, deliver to the agency directed by the NIST representative for the calibration.
- The vendor shall ascertain and certify that the equipment is functioning appropriately. Notification should be given if repairs or replacement are required before service is performed and before instrument is returned for use.
- Calibration and maintenance records for NIST certified equipment are to be maintained.
- It may be more cost effective to replace NIST certified timers at the end of their current certificate rather than to send for recalibration.

REFERENCE(S):
OSBI CSD Quality Manual
ISO/IEC 17025:2005
ASCLD/LAB-*International* Supplemental Requirements – Testing
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_7 Thermometers (top ↑)

7.1 Performance Check

- An annual performance check will be performed on each NIST-traceable thermometer used for conducting performance checks.
- If the performance check is not completed within the anniversary month of the last check, the thermometer shall be removed from casework until a performance check is successfully completed.

7.2 Calibration

- Set a water bath to the desired temperature.
- Allow water to heat for at least 30 minutes.
- Place bulb of each thermometer to be checked into the water bath along with the NIST thermometer.
- Wait several minutes and record the temperature on the Thermometer Calibration Log (QC_Therm).
- Wait 15 minutes and record the temperature again.
- Alternatively, the calibrating digital thermometer can be placed into the same equipment as the thermometer, be allowed to equilibrate, and the reading documented.

7.3 Thermometer Performance

- If thermometer readings equal the NIST calibrated reading, no action is needed.
- If the NIST thermometer measurement is within ± one (1) degree of the thermometer being tested, then the amount of deviation shall be noted and all other readings adjusted by the same amount, and in the same direction. If deviation of more than ± one (1) degree is observed the thermometer shall be replaced or taken out of service.

ATTACHMENT(S):
FBU Policy Manual QC_Therm

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_8 Balances (top ↑)

8.1 Routine Maintenance

- To keep the balances operating properly, the housing, chamber and pan should be kept clean and free from foreign material.
- If necessary, a cloth dampened with a mild detergent may be used.

8.2 Performance Check

- Balances shall be subjected to a performance check annually.
- If the performance check is not completed within the anniversary month of the last check, the balance shall be removed from casework until a performance check is successfully completed.
- Performance checks will be performed by an outside vendor and this check will be documented using QC_EquipMain.

8.3 Balance Performance

- If any balance fails to perform within the accepted range, troubleshooting procedures provided in that individual model's instructions and operations manual should be followed.
- If problems persist that cannot be corrected in the laboratory, the balance shall be serviced by an authorized service center.
- Certification from the external vendor shall serve as the documentation for the successful completion of the performance check.

ATTACHMENT(S):
FBU Policy Manual QC_EquipMain

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_9 Thermal Cycler Temperature Verification Systems (TVS) (top ↑)

9.1 Performance Check

- The OSBI Forensic Biology Units shall perform an annual performance check consisting of obtaining an external vendor certification for each TVS used to complete the performance checks for the thermal cyclers.
- If the performance check is not completed within the anniversary month of the last check, the TVS shall be removed from service until a performance check is successfully completed.

9.2 TVS Performance Check Documentation

- Certification from the external vendor shall serve as the documentation for the successful completion of the performance check.

REFERENCE(S):
OSBI CSD Quality Manual
ISO/IEC 17025:2005
ASCLD/LAB-*International* Supplemental Requirements – Testing
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_10 7500 Real-Time PCR Systems (top ↑)

10.1 Maintenance Schedule

10.1.1 Monthly

- Perform the instrument function test
- Record lamp hours
- Perform a background calibration

10.1.2 Every 6 months

- Perform an ROI calibration
- Perform an optical calibration
- Perform a Pure Dye Spectra calibration

10.1.3 As Needed

- Restart the computer
- Defragment the computer hard drive
- Clean the sample wells
- Replace the instrument fuses
- Replace the halogen lamp (~2000 hours)
- Note: After replacing the halogen lamp, the following calibrations must be performed in this order: ROI ► Background► Optical► Pure Dye► Instrument Verification

10.1.4 Documentation of Scheduled Maintenance

- Record all performed tasks on the 7500 Real-Time PCR Instrument Maintenance and Calibration Log (QC_7500).

10.2 Performance Check

- A performance check will be completed annually on the 7500 Real-Time PCR Instrument.
- If the performance check is not completed within the anniversary month of the last check, the 7500 Real-Time PCR Instrument shall be removed from casework until a performance check is successfully completed.
- The annual preventative maintenance (PM) performed by the vendor will serve as the annual performance check.

10.3 Performance Check following Repair, Service, or Calibration

- Certification from the external vendor shall serve as the documentation for the successful completion of the performance check following repair, service, or calibration.

ATTACHMENT(S):
FBU Policy Manual QC_7500

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_11 9700 Thermal Cyclers (top ↑)

11.1 Performance Check

- An annual performance check on all 9700 thermal cyclers will be performed using a Temperature Verification System (TVS) [refer to 9700 User's Manual in the attachment of the FBU Policy Manual].
- If the performance check is not completed within the anniversary month of the last check, the 9700 Thermal Cycler shall be removed from casework until a performance check is successfully completed.
- The performance check will be performed using the Temperature Verification function under Diagnostics in the 9700 Thermal Cycler Software.

11.2 Performance Check following Repair, Service, or Calibration

- A performance check will be completed using a Temperature Verification System (TVS) after any repair, service, or calibration and before using in casework.

11.3 9700 Thermal Cycler Instrument Performance

11.3.1 Temperature Calibration Verification test

- If the block average is more than 1.0°C above or below the 45°C point or more than 1.0°C above or below the 85°C point, the test is to be run two more times.
- If the thermal cycler meets standards two of three attempts, then it shall be deemed acceptable.
- Record results on QC_9700_0.5 or QC_9700_0.2 as needed.

11.3.2 Temperature Uniformity Test

- If the results of either calculation are greater than 1.0°C, the test is to be run two more times.
- If the thermal cycler meets standards two of three attempts, then it shall be deemed acceptable.
- Record results on QC_9700_0.5 or QC_9700_0.2 as needed.

11.3.3 If a 9700 thermal cycler fails to perform correctly, the instrument shall be removed from service and a qualified service technician shall be contacted.

ATTACHMENT(S):
FBU Policy Manual QC_9700_0.2 FBU
Policy Manual QC_9700_0.5

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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QC_12 3500 Genetic Analyzers (top ↑)

12.1 Individual Steps of the Procedure

The following outlines the schedule for the specific maintenance tasks needed to ensure optimal performance of the 3500 Genetic Analyzer and computer. Daily, Every Other Day, and Weekly tasks can be performed less often if the instrument is not in use, as long as the tasks are performed before the next use. Maintenance should be documented on QC_3500_Main.

12.1.1 Daily when Instrument is in Use

- Check Dashboard
- Visually inspect the fluid level in the buffer containers

12.1.2 Weekly (7 days)

- Check storage condition of used arrays to ensure array tip is covered in the reservoir
- Record number of runs
- Run Wash Pump and Channels Wizard
- Replace Anode and Cathode Buffer
- Replace septa on reservoirs
- Replace POP-4 pouch

12.1.3 Monthly

- Flush the Pump Trap
- Empty the condensation container and water trap waste container

12.1.4 Quarterly

- Archive the Library to the network
- Run a HID Install Standard Performance Check

12.1.5 As Needed

- Restart the computer
- Install new capillary array: Capillary arrays can be used until undesirable data such as poor sizing precision, poor allele calling, poor resolution, or decreased signal intensity is consistently observed.
- Perform a Spatial calibration after replacing a capillary array, when a service engineer performs an optical service procedure such as realigning or replacing the laser, CCD cameras or mirrors, when you open the detector door or move the detection cell, or when you move the instrument.
- Perform a Spectral calibration if you change dye sets, change the capillary array, change polymer type, when a service engineer performs an optical service procedure such as realigning or replacing the laser, CCD cameras or mirrors or when you see an increase of spectral separation (pull-up/pull-down peaks) in the raw or analyzed data. Place this document in the desktop folder on the instrument computer.
- Defragment the computer hard drive.

12.2 Quarterly Performance Check

- A **HID install standard performance check** will be performed for all 3500 Genetic Analyzers to verify the instrument conforms to analysis sizing precision, sizing range and peak height specifications at least **quarterly**.
- If the performance check is not completed before the end of the quarter, the 3500 Genetic Analyzer shall not be used for casework until a performance check is successfully completed (only takes about 30 minutes).
- Document results of the performance check by saving it to the appropriate network folder.
- Place this document in the desktop folder on the instrument computer.

12.3 Performance Check following Installation of Capillary Array or Repair, Service or Calibration

- A **HID install standard performance check** will be performed for all 3500 Genetic Analyzers to verify the instrument conforms to analysis sizing precision, sizing range and peak height specifications after the replacement of the capillary array or after repair, service or calibration. This performance check can also serve to satisfy the as the quarterly performance check requirement above.
  - If instrument sensitivity concerns are present, a dilution series shall be analyzed at the instruction of the Technical Manager.
  - Document results of the performance check by saving it to the appropriate network folder.
    NOTE: If a Preventive Maintenance visit from the manufacturer was performed which did not require any alterations of the optics of the instrument (i.e. laser), then a performance check of the instrument consisting of an evaluation of the first positive and negative controls ran on the instrument will be performed and documented in the maintenance notebook form QC_3500_Main

before any results from the run will be reported. If the optics are adjusted, a HID install standard performance check will be successfully completed before casework.

12.4 3500 Genetic Analyzer Performance

- If a 3500 Genetic Analyzer fails to perform correctly, the instrument shall undergo troubleshooting to the extent of the technical skills of the laboratory personnel an attempt to re-establish satisfactory performance.
- If the 3500 Genetic Analyzer continues to yield unsatisfactory performance, the instrument shall be removed from service and a qualified service technician shall be contacted.

ATTACHMENT(S):
FBU Policy Manual QC_3500_Maint_Log
FBU Policy Manual QC_EquipMain

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_13 Pipettors (top ↑)

13.1 Performance Check-Annual

- An annual performance check will be done on all pipettors (mechanical pipettes). If the performance check is not completed within the anniversary month of the last check, the pipettor shall be removed from casework until a performance check is successfully completed.

13.2 Pipettor Type Requirements

- Adjustable pipettors shall be checked at the minimum and maximum values, with the exception of the 2 µL and 10 µL pipettors, which will be checked using 1 µL for the minimum value. For Rainin pipettors, the range for P-2 is 0.1 to 2 µL, P-10 is 0.5 to 10 µL, P100 is 10 to 100 µL, P-200 is 50 to 200 µL and P-1000 is 100 to 1000 µL.
- Repeat pipettors shall be checked at the minimum and maximum values based on the dispensing volume range associated with the attached tip. In addition, repeat pipettors will be checked at the aliquot volume desired. Repeat pipettors shall be labeled with the approved volume range and aliquot volume.
- Multichannel pipettors shall be checked at the minimum and maximum values with each channel being checked individually and the set of channels being checked collectively.

13.3 Individual Steps of the Performance Check

- Using a calibrated balance, place a weighing receptacle on the balance and tare.
- Deliver the desired volume of water to the weighing receptacle and record registered weight. At the FSC, this may be accomplished by using the Calibri software recording the measured volume.
- Calculate % error for each measurement using the formula: (Delivered weight - Expected weight) / Expected Weight x 100%, if taking measurements by hand.
- Document results for each pipettor on the Pipettor Calibration Log (QC_PipetCal) or archive the "Calibration Report" from the Calibri software in the appropriate QC folder on the network.

13.4 Pipettor Performance

- Determine whether the pipettor passed or failed the calibration. The tolerance for pipettor volumes greater than or equal to 10 µL is 3%. The tolerance range for volumes less than 10 µL is 10%.
- If the percent error is greater than that stated above, repeat measurement four more times. Average the volume of the five measurements and calculate the percent error using the Pipettor Calibration Reproducibility Log (QC_PipetCalReprod). If the percent error is within the established tolerance,

the pipettor is accepted as meeting criteria.

- If the pipettor fails after 5 tries, adjustment/maintenance is necessary. This can be done in house or at an authorized service center.
- Certification from the external vendor shall serve as the documentation for the successful completion of the performance check. The calibration certificates issued by these competent calibrating laboratories shall contain the measurement results, including the measurement uncertainty and/or a statement of compliance with an identified metrological specification.
- NOTE: Calibration labs fulfilling the requirements of ASCLD/LAB ISO 17025 are considered to be competent. Copies of ISO 17025 certificates shall be maintained with the calibration/test reports as sufficient evidence of traceability of the calibration data reported. A review of the certificate(s) by the TM (or designee) shall serve as documentation of this check of compliance regarding function and calibration.

ATTACHMENT(S):
FBU Policy Manual QC_PipetCal
FBU Policy Manual QC_PipetCalReprod

REFERENCE(S):
OSBI CSD Quality Manual
ISO/IEC 17025:2005
ASCLD/LAB-*International* Supplemental Requirements – Testing
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_14 Automate Express™ Forensic DNA Extraction System

14.1 Maintenance Schedule

The maintenance tasks necessary to ensure optimal performance of the Automate Express™ are detailed below and shall be performed as per the instrument user guide.

14.1.1 Daily

• Clean piercing units with alcohol

14.1.2 Biweekly

• Apply silicon grease to the D-rings of the nozzle

• Clean the cartridge rack, tip and tube rack, and magnets with a mild detergent allowing the parts to dry before use

14.1.3 Monthly

• Perform axis and temperature tests

14.1.4 Annually

• Replace the D-rings

• Planned maintenance will be performed by a qualified service technician.

• An annual performance check is required and can be achieved by the planned maintenance of the instrument. If a PM is not performed, QC of a new PrepFiler® Express kit using two NIST traceable samples along with the appropriate documentation will serve as the performance check. These samples must achieve the expected quant results.

14.1.4 As Needed

• Clean the cartridge rack, tip and tube rack, and magnets with a mild detergent allowing the parts to dry before use

• While the instrument is off, remove and clean bottom tray with mild detergent and dry before use

• If an instrument must undergo repair, service, or calibration a performance check is required with the running of two NIST traceable samples. These samples must achieve the expected quant results.

NOTE: Do not use acid or bases (bleach) to clean the instrument.

14.2 Documentation of Scheduled Maintenance

• Record all Biweekly, Monthly and Annual tasks performed on the Automate Express™ Maintenance and Calibration Log (QC_Automate_Maint_Log).

ATTACHMENT(S):
FBU Policy Manual QC_Automate_Maint_Log

FBU Quality Control Procedures

REFERENCE(S):

OSBI CSD Quality Manual
ISO/IEC 17025:2005
ASCLD/LAB-*International* Supplemental Requirements – Testing
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

QC_15 Thermomixers (top ↑)

15.1 Cleaning

- Use neutral cleaning agents to clean the thermomixer housing and thermoblocks as needed.
- Ensure that all parts are dried thoroughly before use.

15.2 Performance Check - At a minimum, the OSBI Forensic Biology Unit shall perform an annual performance check for each thermomixer consisting of both a temperature and timer verification. If the performance check is not completed within the anniversary month of the last check, the thermomixer shall be removed from service until a performance check is successfully completed.

15.2.1 Temperature verification

- Remove the 0.02mL well cover located in the middle of the smart block.
- Verify the appropriate temperatures used in policy (See DNA_1 and DNA_2) with a Temperature Verification System (TVS).
- Document the results on the Thermomixer Calibration Log (QC_Thermomixer).

15.2.2 Timer Calibration

- Measure the timer against a stopwatch or other calibrated timer set for the same time interval(s).
- Document timer calibration results on the Thermomixer Calibration Log (QC_Thermomixer).

15.3 Thermomixer Performance

15.3.1 Temperature

- If the temperature is more than 1.0˚C above or below the desired temperature, the test is to be run two more times.
- If the temperature verification fails two of the three times, the thermomixer shall be removed from service and a qualified service technician shall be contacted.

15.3.2 Timer

- If the timer varies more than 30 seconds in five minutes, the instrument shall be removed from service and a qualified service technician shall be contacted.

ATTACHMENT(S):
FBU Policy Manual QC_Thermomixer

REFERENCE(S):
FBI's Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories
FBI's Quality Assurance Standards (QAS) for DNA Databasing Laboratories

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History

Revision # (Effective Date)	***History Description*** (**top ↑**)
Revision 0 (11-02-2010)	Original document with a combined protocol format.
Revision 1 (05-15-2011)	Annual Review. Incorporate deviations as follows: Addition of Language for Disposition of Serology Work Product (dated 01-14-11) Addition of Report Language for CODIS Entry (dated 11-22-10) Allowance of additional washes with Microcon device (dated 11-22-10) Addition of AR/TR Supplemental Requirements (dated 11-04-10) Authorization to Amplify <0.5ng without needing TM Approval (dated 11-09-10) Changes to Report Wording for CASE_7 AP Spot Test (dated 02-11-11) Changes to FBU Organizational Chart to reflect Personnel Changes (dated 12-06-10, 03-08-11) Modifications to Procedure for SER_3 Human Blood Testing (dated 12-17-10) Additional of Thermal Cycler Asset Number to PCR Setup Worksheet (dated 11-04-10) Clarification added for the Treatment of Reagent Blanks (dated 03-09-11) Clarification added for QM_9 Analytical Procedures regarding Reagent 26-11) Other changes made include: Addition of wording DNA data evaluation pursuant to the OSBI FBU Internal Stochastic Threshold Validation Clarification added for SER_7 Hair Analysis regarding allowance to test only select apparent animal hairs based on training and experience Report Wording for Criminal Paternity cases clarified Clarification to QM_9 Analytical Procedures regarding annual NIST requirements Addition of “within the anniversary month” for all annual QC requirements Addition of Report footer to include disclaimer regarding “…results, opinions and interpretations of analyst whose name appears on the report” Addition of Stochastic Threshold Validation information throughout document.

Minor grammatical corrections for consistency throughout document.

Added "AΩ" to the footer of all Management Forms, in accordance with OSBI CSD QP 2 – Document Control

Incorporation if SWGDAM Guidelines for Autosomal STR Typing for Forensic Testing Laboratories into the DNA Interpretation and Statistical Analysis sections.

Revision 2 (05-15-2012) Annual Review.

Incorporate deviations as follows:

Establish definition of "reagent" in FBU QM_2 Definitions.

Omit requirement for multiple reagent blanks to be created and combined when multiple same samples are being extracted and combined in the same batch is not necessary, per FBI clarification [FBU QM_9.5.1.8]

Changed "should" to "shall" to conform out policy to QAS Standards [FBU QM_13.1.7]

Add requirement to document sperm search slide number/well in case notes in step 12 [FBU SER_6]

Change wording regarding the documentation of hair analysis [FBU SER_7]

Change 100% ethanol to a grade of equal to or greater than 95% [FBU DNA_1]

Account for use of in-house spreadsheet (OSBI STATS) for calculation of statistics where heterozygosity is in question ($2p-p^2$) [FBU CASE_6]

Incorporate two analysis methods to account for 5 and 10 second injection times for Identifiler Plus casework [FBU DNA_8]

Change the peak amplitude thresholds to coincide with the interpretation thresholds established during our internal peak stochastic validation [FBU DNA_8.2]

Changed "minus stutter ratio" on the allele tab to "minus stutter distance" for both OSBI Casework_ID Plus method and OSBI Casework_YFiler method [FBU DNA_8.2.4]

Add requirement to forward profiles from contamination not attributed to samples in the batch or OSBI employees to the CODIS Unit for evaluation for entry into the Contaminant Index (SDIS only) [FBU CASE_4.7].

Remove reference to FBI's Popstats program to calculate the random match probability of a profile due to incorporation of the OSBI STATS program [FBU CASE_6.1]

Incorporation of the use of family relatedness statistics [FBU CASE_6 and CASE_7]

Removed wording "The source of the Known from…" [FBU CASE_7.7.9, 7.7.10, 7.7.12]

Remove requirement to change electrode storage solution from monthly maintenance and add it to the "as needed" maintenance [FBU QC_2]

Remove requirement for quarterly download for 7500, 310 and 3130 instruments and require this data to be placed onto the server at (\\Pm-fsc13000s\dna_data) once complete. Requirement for defragmenting of the hard drives of the 7500, 310 and 3130 was removed and should be done annually or as needed (if the computer is running slowly, etc.) [FBU QC_10.1.3, QC_12.1.5 and QC_13.1.6]

Clarification added as to where to find the procedure for the execution of the quality check for the 9700 thermal cycler [FBU QC_11]

Remove requirement for weekly restart of instrument and computer [FBU QC_13.1.3]

Updated organizational chart for the discipline [FBU QM_OrgChart] and Abbreviation List [FBU QM_Abbrev]

Changed references of QAS Standards for Forensic DNA Testing Laboratories, effective date of July 1, 2009 to effective date of September 11, 2011 [entire document]

Changed SER_6 Microscopic Identification of Spermatozoa to reflect new procedure using Phosphate Buffered Saline and Permount.

Reorganization of the numbering in the Quality Manual section to align with the Quality Assurance Standards.

Revision 3 (11-01-2013) Annual Review.

Incorporate deviations as follows:

Allow for p30 extract storage for up to 5 days and the use of Phosphate Buffered Saline in conjunction with a 30 minute extraction time [FBU SER_5]

Remove requirement for weekly restart of 7500 computers [FBU QC_10.1.1]

Change the purification procedure due to new nylon membranes in Microcon filters [FBU DNA_1.5.2 and DNA_2.5.2]

Allow analysts the discretion to add an additional TE^{-4} wash of 200-400µl of TE^{-4} and a 20 minute spin to samples (and an associated reagent blank) for the purpose of removing possible inhibition prior to the quantitation step [FBU DNA_1.5.2 and DNA_2.5.2]

Allow analysts the discretion to add an additional PCI step to samples (and an associated reagent blank) for the purpose of removing possible inhibitors prior to

quantitation step [FBU DNA_1.5.2 and DNA_2.5.2]

Allow for Outsourcing of cases worked with Cold Case Grant funds (FBU QM_17]

Add "PBS" as an accepted abbreviation [FBU QM_Abbrev]

Updated organizational chart for the discipline [FBU QM_OrgChart]

Add Requirements for Controls listed in Case Record and clarify that the controls must be ran and recorded (entire document)

Add lists of attachments and references in each section, as applicable (entire document)

Modify references to OSBI CSD Quality Manual sections to account for revision of Quality Manual (entire document)

Add definition of Low Copy Number (LCN) DNA Analysis [FBU QM_2]

Clarify Permount must be used in a hood [FBU SER_6]

Add requirement for clarification of sample naming if a sample is re-ran multiple times within a GeneMapper project [FBU DNA_8]

Add requirement for note-taking in the BEAST [FBU CASE_1]

Add requirement for circling or underlining of case number for each page in case file (if batching) since highlighting will not replicate when imaged/archived [FBU CASE_1]

Add clarification that DNA data will be placed on DNA server for storage [FBU CASE_1]

Add option for re-injection of sample with quantitation result producing no result at genetic analysis [FBU CASE_4]

Modify report wording making some DNA wording optional to decrease in complexity of report [FBU CASE_7]

Add clarification that "QUICK SPIN ONLY" centrifuges do not require annual performance checks [FBU QC_5]

Add requirement that annual balance performance check performed by outside vendor be documented on the FBU QC_EquipMain worksheet [FBU QC_8]

Add clarification that vendor calibrating pipettors must be ISO accredited if the external vendor calibration certificate is to suffice for verification of the required performance check [FBU QC_14]

Add wording to allow determination of major/minor mixture with technical reviewer agreement [FBU CASE_5]

Remove requirement for case number in "Comments" field of Quantifiler setup – this information is in the data file and the analysis print-out. [FBU DNA_3]

Remove requirement for duplicate quantitation of samples [FBU DNA_3]

Adjust FBU DNA_Quant worksheet to reflect single v. duplicate quantitation [FBU DNA_Quant worksheet]

Removal of references to 310 and 3130 Genetic Analyzers (entire document)

Add use of 3500 and GeneMapper ID-X (entire document) as well as Peak Stochastic Threshold Validation information

Revision 4 (09-01-2014) Annual Review.

Incorporate Deviations as follows:

Remove daily requirement to document "Check Consumables on Dashboard" on the Maintenance Form (QC_3500_Maint Form) and add "Archive Library" as a quarterly requirement to the form [QC_12 and QC_3500 Maint Log]

Change wording regarding certificates of calibration for pipettors in QC_13.4

Add AutoMate Express Instrument using PrepFiler® Express and PrepFiler® Express BTA forensic DNA extraction kits to the policy manual in necessary sections [DNA_1 and DNA_2 and associated worksheets]

Add Robotic Systems to list of critical equipment in QM_10.4.1 and add "robotic systems" to the list of critical equipment needing a performance check after repair, service and/or calibration

Remove "currently the OSBI does not use automated methods for casework" from section QM_13.1.1

Change wording in CASE_7.7.19 to "The DNA profile(s) from Item(s) (list item numbers) have been entered into the CODIS database." To reflect change in workflow between casework and CODIS.

Modify DNA_6.4.3 to remove requirement to enter the plate name into the "Run Name" field.

Remove reference to "plate record" in CASE_1.4.6.

Add language to DNA_7.2.7 to make sample and project naming more consistent among analysts.

Add language to CASE_1.2.2 addressing requirement for headers on electronic case files.

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Add language to CASE_1.4.5 stating that all other electropherograms besides original 15 second injection must contain labeling indicating their injection order.

Add wording to QM_12 stating that analysts will route their technical reviews to an assortment of analysts (as available) in a rotation to ensure that all analysts are reviewing one another's casework.

Add "and attachments" to QM_1.5.

Remove reference to Trace Evidence Unit protocol "Portable Hood Use".

Add language to QM_5.2.4 referencing "performing DNA analysis".

QM_6.3 remove "three".

Grammatical and formatting corrections.

Add clarification about use of the BEAST for note taking and that the PDF portfolio is the final case record [CASE_1]

Add option for footnote in reports explaining p30 [CASE_7.5]

Remove FBU QM_ARTR form since it is required to use the BEAST-generated form for all needs of a hard-copy AR/TR form per OSBI CSD QP 31 – Reviews.

Add wording to QM_12.4 to include requirements in OSBI CSD QP 31 – Reviews for documenting corrections needed during review process.

Add requirement for comma in reporting DYS385 results with two alleles to CASE_1.4.7.

Add language in QM_5.6.1 regarding degree and experience requirements for Laboratory Technicians.

Add statement to page 2 of Manual addressing electronic transmission of DNA profiles per QAS.

Add language in CASE_1.1.12 addressing requirement to obtain and maintain documentation granting permission for consumption from requesting agency or laboratory for retention in our case record. Referenced this requirement in QM_7.3.2 as well.

Add language in QC_13.2 for check of aliquot volumes of repeat pipettors.

Add requirement to CASE_1.4.6 for DNA data retention per CSD Quality Manual section 16.2, II, A., 2, c.

Updated definition of "Off-Scale Peaks (OS)" to match GMID-X User's Manual

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Revision 5 (11-01-2015) Annual Review.

Incorporate Deviations as follows:

QC_12 – change requirement for recording number of runs from daily to weekly while instrument is in use.

CASE_1.1.1 Change “folders” to “headers”.

CASE_1.4.6 Change “on” to “in” and “BEAST notes” to “BEAST case record”.

Addition of acceptable range of results as required per the QAS Standard 9.3 discussion section for the forms listed here: QCR_DNA_AutoMate_Ecell_Digest_Buffer, QCR_DNA_AutoMate_EXTReagents, QCR_DNA_PrepFiler_Kits, QCR_DNA_QUANTKIT.

QCR_SER_RSID form modified to include a space for the lot numbers and expiration dates of the extraction buffer and running buffer in the RSID-Blood kits.

QM_13 Proficiency Testing wording modified to add clarification regarding proficiency tests and to include Technician tests.

QM_OrgChart updated to reflect movement in personnel.

CASE_6.1 changed “computer program” “allele frequencies”.

Modified CASE_5, CASE_6, and CASE_7 after completion of supplemental in-house mixture validation.

Changed “may rename stutter” to “will rename stutter” in CASE_5.2.3.1.

Added “is acceptable for determining whether a peak is off-scale” to CASE_5.2.3.8 (inadvertently deleted in Rev 3 and Rev 4).

Changed “can be excluded” to “is excluded” in CASE_7.7.3.2.

Modified naming of attachment titles for SWGDAM Y-STR document.

Add 1.0M DTT preparation and storage details to QCR_DNA_AutoMate_EXTReagents form.

Add language regarding property crimes with no listed subject do not require prior written authorization from the District Attorney’s Office prior to consuming biological evidence, but clarify that major (persons) crimes and property crimes with a listed subject do require written authorization prior to consuming evidence to CASE_1.1.12.

CASE_4.1 Added in policy wording that swabs from the same area/staining with differing serology results may be combined for DNA analysis.

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Incorporate Thermomixer QC [QC_15 Thermomixers].

Add Pass/Fail column to QC_2 pH Meter Form based on internal audit suggestion.

Add “Screen Filter Cleaning” column to QC_H2O based on internal audit suggestion.

Modify references to “CODIS Policy and Procedure Manual” to “CODIS Policy Manual”.

Changed allowance of batch GMID-X project “at analyst’s discretion” to “with prior Technical Manager approval” in DNA_7.

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Approval

(**top ↑**)

Per OSBI CSD QP 2 – Document Control, technical protocols/procedures, discipline quality manuals, and related attachments and references will be approved by the appropriate Technical Manager and the OSBI CSD Director or designee.

FBU Technical Manager		10/22/2015
	J. Janice Joslin	Date
CSD Division Director		10/28/2015
	Andrea R. Swiech	Date