OSBI Forensic Biology Units' Policy Manual for Casework Analysis

Revision 15

Effective July 07, 2023

Conforming to the International Standard ISO/IEC 17025:2017 Program of Accreditation & the Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories

ATTENTION:

If any portion(s) of this policy manual is/are unclear to any analyst/technician or if a circumstance arises outside the scope of this document, it is the responsibility of each individual to notify the technical manager and the respective supervisor immediately to seek clarification/guidance and obtain approval, if necessary, regarding the issue before proceeding.

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Other OSBI Laboratory Procedure References:
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI CODIS Unit Policy Manual

OSBI Trace Analysis Protocol Manual: Polarized Light Microscope (PLM) & Collection of Trace Evidence

Quality Manual

CWQM_1 SCOPE & APPLICABILITY (↑ Table of Contents)

1.1 The Oklahoma State Bureau of Investigation (OSBI) Criminalistics Services Division (CSD) includes the Forensic Biology Discipline, which encompasses multiple forensic biology units and the Combined DNA Index System (CODIS) Unit.

1.2 The OSBI Forensic Biology Units' Policy Manual shall apply to work specifically performed by analysts/technicians and support staff working within officially defined forensic biology units or by analysts/technicians and support staff working in the capacity of a forensic biologist or associated technician.

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 OSBI and to ensure the quality and integrity of the data generated by the units. The OSBI CODIS Unit maintains a separate quality manual, which can be found in the OSBI CODIS Unit Policy Manual.

1.4 Unless specifically stated, the Quality Manual (QM) of this document only applies to laboratories performing forensic DNA testing (DNA casework) or utilizing the Combined DNA Index System (CODIS).

1.4.1 Forensic DNA testing begins at sample extraction or direct amplification.

1.4.2 If applicable, these requirements apply to forensic DNA testing laboratories using Rapid DNA instruments/Systems on casework reference samples. The use of Rapid DNA instruments/Systems is not approved for use on forensic samples.

1.5 For the purposes of audit and accreditation processes, laboratories shall have available objective proof, such as written documentation, of satisfying applicable requirements and demonstrating compliance.

1.6 Hyperlink and attachment maintenance, such as the removal, addition, and/or correction of hyperlinks/attachments, shall not constitute a new document revision.

Attachment(s):
None

Reference(s):
OSBI CODIS Unit Policy Manual

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

CWQM_2 DEFINITIONS (↑ Table of Contents)

Terms in this policy manual follow the definitions set for in Standard 2 of the QAS. In addition, the OSBI forensic biology units define the additional following terms as having the meanings specified:

Additive stutter (AST) is a combination of plus and minus stutter. See **Stutter (ST)** below.

Allele or true allelic peak is 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.

Amplification set may contain samples and reagent blanks from different cases and/or questioned and known samples together.

Analytical threshold (AT) is 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. It is the minimum height requirement, determined through validation testing, at or above which detected peaks/signal can be reliably distinguished from background noise; peaks/signal at or above this threshold are generally not considered noise and are either artifacts or true alleles.

Annual is once per calendar year.

BEAST is the Barcoded Evidence Analysis Statistics and Tracing program within the OSBI Laboratory Information Management System (LIMS).

Concurrent is taking place at the same time with the same conditions.

Differential extraction is the process for extracting DNA from all samples testing presumptive or confirmatory positive for seminal fluid.

DNA analysis is using the Polymerase Chain Reaction (PCR) to examine Short Tandem Repeats (STR) utilizing capillary electrophoresis; in accordance with the QAS, DNA analysis begins at sample extraction or direct amplification.

Dye blobs are disassociated primer dyes that are reproducible artifacts of the PCR process and often observed with a particular lot of amplification kits. Although it is not entirely clear why dye blobs occur, they are believed to be caused when the fluorescent dye tags attached to the amplification kit primers begin to break down over time. The dissociated primer dyes are carried through the PCR amplification step and when injected onto the capillary array can appear as peaks during data analysis. However, dye blobs are usually wider than true allelic peaks and are typically seen in one color although can also be see in multiple dye colors.

"End date" of analysis is the date a report of analysis is approved by the examining analyst in the BEAST system.

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

Forensic Genetic Genealogy (FGG)/Forensic Genetic Genealogical DNA Analysis and Search (FGGS) or investigative genetic genealogy combines law enforcement's use of DNA analysis with traditional genealogy research to generate investigative leads for unsolved violent crimes. A vendor laboratory may perform forensic genetic genealogical DNA analysis for a forensic or reference sample of biological material to develop a FGG profile, which can subsequently be searched in a publicly-available open-data personal genomics DNA database or a direct-to-consumer genetic genealogy service (i.e.

third-party SNP databases). The FGG profile is compared by automation against the genetic profiles of individuals who have voluntarily submitted their biological samples or entered their genetic profiles (i.e. service users). A computer algorithm is used to evaluate potential familiar relationships between the (forensic or reference) sample donor and service users. Relationships can then be evaluated using traditional genealogy research in an effort to generate an investigative lead.

Instrument baseline threshold is the RFU value below which a peak is indistinguishable from the baseline noise.

Instrument spike (SP) is a non-reproducible artifact that occurs occasionally within an electrophoretic run resulting in the appearance of an anomalous peak(s) on the electropherogram. A peak resulting from an instrument spike is easily identified upon close examination; such a peak will usually be composed of all colors with approximately equal apparent fluorescent intensity and occur at the same data point and/or scan number in GeneMapper® *ID-X* (GMID-X). Spikes are believed to be caused by scattering of incident light due to the passage of dust particles through the excitation laser beam.

Intimate sample is a sample originating/taken directly from an individual's body, such as body swabs (e.g. vaginal swabs, breast swabs, neck swabs, penile swabs, swab of a hand, etc.), fingernails, etc. An intimate sample is expected to yield the DNA profile from the individual the sample is taken from.

Limit of detection (LOD), as it pertains to genetic analysis, 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 by the OSBI, is 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 and/or voltage outside the scope of the OSBI's internal validations 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 are alleles that contain an incomplete repeat unit (e.g. "9.3" at TH01).

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

Mixture is when two or more contributors are present in a DNA profile.

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

Off-scale peak (OS) is a reproducible peak above the linear detection of the instrument or any fluorescence signal that exceeds the detection threshold of the instrument. The OS qualify 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) is 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) is the RFU value above which it can be assumed that peak dropout does not occur and any observed allelic peak that has no sister allele present can reasonably be believed to be homozygous. The peak stochastic threshold for an amplification kit is the RFU level 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 that affects the quality of the amplification process.

Process step refers to each step in a process of analysis. This would include, but is not limited to, adding protocol specific reagents at listed volumes or use of any other equipment in the analysis process, and, as such, an associated reagent blank must also be taken through the process concurrent to any samples. However, in some process steps, such as concentrating 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) is 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.

Quantification/Quantitation[1] 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. It may also be referred to as a "Non-template control (NTC)."

Reagent is any substance or mixture used in the analysis process of the Forensic Biology Discipline. Chemicals used to make reagents are not considered "reagents," but rather chemicals (e.g. NaOH). Reagents will have an expiration date of one year from the date of receipt, unless otherwise specified by the manufacturer or in the OSBI Forensic Biology Units'/CODIS Policy Manuals and/or their attachments.

Review date is the completion date of the review of the protocols.

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

Single-source is when one donor/contributor is present in a DNA profile.

Single nucleotide polymorphisms (SNP) are DNA sequence variations that occur when a single nucleotide (A, T, G or C) in a genomic sequence is altered. The variations may be used to distinguish people for the purposes of biological relationship testing, such as with forensic genetic genealogy.

Standard extraction is used for all samples, except those requiring a differential extraction.

"Start date" of analysis is the date an assignment is made to an analyst in the BEAST system.

Stutter (ST) is a reproducible artifact of the PCR process. It is 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 is the equivalent to "Technical Leader" as defined by the QAS.

Trailing primer peak (TP) is a peak or peaks associated with the primer peak that fall within the size calling range.

Trainee is an analyst or technician undergoing training in a particular area (e.g. serology or DNA analysis).

Trisomy is three alleles at a single-source STR locus.

Virtual allele is an allele that is labeled by the software even though it is not present in the allelic ladder. For example, if using the GlobalFiler™ 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

[1] Terms may be used interchangeably throughout this document.

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 is the material utilized in testing and/or generated as a function of the analysis process that is not subject to a chain of custody.

Working volume is 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 a 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 QAS.

Year refers to calendar year.

Attachment(s):
None

Reference(s):
FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_3 QUALITY ASSURANCE PROGRAM (↑ Table of Contents)

3.1 **Quality System**

The OSBI Criminalistics Services Division (CSD) has established and maintains a documented quality system, which includes a general, unit-neutral quality manual applicable to all members of the OSBI CSD. The forensic biology units have further established and maintain this documented unit-specific quality manual to specifically address the quality system applicable to the testing activities performed by the respective units for forensic serology and forensic DNA testing.

3.1.1 The quality system will address the following elements:

3.1.1.1 Goals and objectives

3.1.1.2 Organization and management

3.1.1.3 Personnel

3.1.1.4 Training

3.1.1.5 Facilities and evidence control

3.1.1.6 Validation

3.1.1.7 Analytical procedures

3.1.1.8 Equipment

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 Professional development

3.1.1.15 Outsourcing ownership

3.1.2 Any document referenced within the quality manual shall be available on-site or be readily accessible (e.g. available online) to each forensic biology casework unit within the OSBI multi-laboratory system.

3.2 **Document Retention**

The forensic biology units shall follow OSBI CSD Quality Procedure (QP) 16.1 (Control of Records) regarding document retention for the following quality and technical records:

- Proficiency tests
- Corrective action
- Audits
- Training records
- Continuing education
- Case files/case records

- Court testimony monitoring

Per the OSBI CSD Quality Procedures, all of the above-listed areas are quality records, except for case files/case records, which are considered technical records.

3.3 **Annual Review of Quality System**

The forensic biology units will 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. This review will be independent of the audit required by Standard 15 (Audits) of the QAS. The review shall be completed under the direction of the technical manager; the completion of the review must be documented and approved by the technical manager.

3.3.1 Forensic Biology Units-Level Documents

3.3.1.1 The annual review of the forensic biology units' quality system may be an ongoing process of incorporating any deviations or suggestions submitted through OSBI CSD QP 3 (Deviations) and/or QP 15 (Preventive Action) into the next revision of the OSBI Forensic Biology Units' Policy Manual. A final review of the incorporated changes will be completed before issuance, and approval will be documented in the Approval section of the policy manual.

3.3.1.2 The technical manager may conduct the annual review by requesting assistance through the Lead Analysts or the Biology Subcommittee, as needed.

3.3.1.3 The final documented approval of the annual review (approved by the technical manager) shall correspond sequentially or identically in date with either the issuance of a new policy revision to the current policy or by the insertion of the "Review Date" and the phrase "Annual Review" within the Policy Manual History.

3.3.1.4 Changes to the policy shall be outlined in the Policy Manual History and attached to the policy manual (**CWQM_Policy_Manual_Hx**).

3.3.2 CSD/OSBI-Level Documents

3.3.2.1 Review of these policies/procedures will be documented in a memo (or other written format), and any proposed deviations/changes will be submitted to the appropriate authority.

3.4 **Annual Review of Case Files**

The forensic biology units will conduct an annual review of case files determined by the technical manager to be a representative sample of cases worked. The scope of this review will be defined and approved by the technical manager prior to each annual review and address both the representative sample and the time period of the case files under review. This annual review may not be replaced by technical reviews as part of Standard 12 (Review) of the QAS. This review will be independent of an external audit conducted under Standard 15 (Audits) of the QAS; however, this review may be conducted concurrently with an internal audit.

3.5 **Goals & Objectives**

The OSBI forensic biology units shall strive to accomplish the following goals and objectives:

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

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

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

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

3.5.5 In accordance with the requirements for federal funding, the OSBI Forensic Biology Discipline will prioritize the analysis of, to the extent practicable and consistent with public safety concerns, samples from homicides and sexual assaults.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **CWQM_Policy_Manual_Hx**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

FBI National DNA Index System (NDIS) Operational Procedures Manual

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

CWQM_4 ORGANIZATION & MANAGEMENT (↑ Table of Contents)

4.1 The OSBI forensic biology units shall have:

4.1.1 A managerial staff with the authority and resources needed to discharge their duties and meet the requirements of the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories.

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 (Nonconforming 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 the Standards.

4.1.2 A technical manager who is accountable for the technical operations. The OSBI Forensic Biology Discipline technical manager is responsible for all technical aspects of the entire OSBI forensic biology multi-laboratory system (discipline), which is comprised of the following laboratories/units, generally handling the case types indicated below, although subject to change, as needed, based on the needs of the OSBI:

- At the Forensic Science Center (FSC):
  - Forensic Biology Unit (FBU): property crimes and major violent crimes other than sexual assault crimes
  - Specialized Forensic Biology Unit (SFBU): sexual assault crimes
  - Cold Case Unit (CCU): cold cases
  - Combined DNA Index System Unit (CODIS): offender/arrestee database samples
- At the Northeast Regional Laboratory (NERL):
  - Forensic Biology Unit (FBU): property crimes and major violent crimes other than sexual assault crimes

4.1.3 A casework CODIS administrator who is accountable for CODIS operations on-site at each individual laboratory facility utilizing CODIS in the OSBI forensic biology multi-laboratory system (discipline).

4.1.4 At least two full-time employees who are qualified DNA analysts at each OSBI laboratory, which are distinct facilities.

4.1.5 The responsibility, authority, and interrelation of all personnel who manage, perform, or verify work affecting the validity of the DNA analysis specified and documented.

4.1.5.1 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 Discipline's chain-of-command. In addition, section CWQM_5 (Personnel) in the quality manual of this document reflects the responsibilities associated with each position within the Forensic Biology Discipline.

4.1.5.2 The organization and management structure of the OSBI CSD is detailed in the OSBI CSD Quality Manual and reflects the interrelation of all appropriate personnel within the CSD. The "OSBI Forensic Biology Discipline Organization & Management Structure" chart (**CWQM_Org_Chart**) is maintained as an attachment to this policy manual and reflects the interrelation of all appropriate personnel within the Forensic Biology Discipline.

4.1.6 A documented contingency plan approved by laboratory management that is followed if the technical manager position is vacated or if the number of qualified analysts falls below two full-time employees who are qualified analysts.

4.1.6.1 The memorandum to the Forensic Biology Discipline titled “Contingency Plan for Technical Manager Vacancy or if Number of Qualified DNA Analysts Falls Below Two” 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.

4.1.6.2 The memorandum to the Forensic Biology Discipline titled “Contingency Plan for Technical Manager Vacancy or if Number of Qualified DNA Analysts Falls Below Two” reflects the plan in the event that a laboratory ceases to have two full-time qualified DNA analysts.

4.2 The OSBI Forensic Biology Discipline uses the date of hire/appointment/promotion as the defined date to use for determining the applicable version of the QAS for requirements to assess education, experience and training of individuals.

4.2.1 If an individual does not change his/her role with a promotion or appointment (e.g. Criminalist I to Criminalist II), then reevaluation of his/her education, experience and training is not required. However, if an individual does change his/her role with a promotion or appointment (e.g. Technician to Criminalist, Criminalist to CODIS Administrator, Criminalist to Technical Manager, etc.), then evaluation of his/her education, experience and training for the new role is required.

Attachment(s):
OSBI Forensic Biology Units’ Policy Manual **CWQM_Org_Chart**

Reference(s):
OSBI Memorandum to the Forensic Biology Discipline: “Contingency Plan for Technical Manager Vacancy or if Number of Qualified DNA Analysts Falls Below Two”

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

FBI National DNA Index System (NDIS) Operational Procedures Manual

CWQM_5 PERSONNEL (↑ Table of Contents)

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. Laboratory personnel shall have the education, training and experience commensurate with the examination and testimony provided.

5.1.1 The responsibilities, duties, and skills required for each of the criminalist (analyst) position classifications (Criminalist I, II, III, IV & V) are outlined in general written job descriptions, which are maintained by the Office of Management and Enterprise Services (OMES) Human Capital Management (HCM) Division of the State of Oklahoma and can be found at: https://pf.payfactors.com/client/job-description-management/job-descriptions?jwt=eyJhbGciOiJIUzUxMiIsInR5cCI6IkpXVCJ9.eyJ0eXBlIjoxLCJjb250ZXh0Ijp7IkNvbXBhbnlJZCI6NzA5MywiSnd0VHlwZSI6MX19.oal6IIIIKWfp48YOCWhoWVkeD_9T1_jflie4r7PY3rDVpyAVsWdfgz7up1Z57rxv7R6vc9kHPZ6QS3Ypdorr4w

Additional selective qualifications for analysts within the OSBI Forensic Biology Discipline are also maintained by the OMES HCM Division and referenced at the end of this policy. Criminalist IV analysts in regional facilities may or may not also serve as DNA analysts.

Job descriptions for laboratory analyst (technician) positions and laboratory support staff positions (e.g. administrative technician) are maintained with the OSBI Human Resources Department.

5.1.1.1 Any analyst who is hired to perform forensic DNA analysis that does not meet the qualifications listed in the general job description for a criminalist position, the selective qualifications for the OSBI Forensic Biology Discipline, or the QAS will be brought into compliance with these requirements prior to commencing any independent forensic DNA analysis.

5.1.1.2 For required higher education classes, a passing grade will be a “C” or higher. QAS-required classes previously completed with a grade lower than a “C” will have to be re-taken. Failure to successfully complete required coursework with a grade of “C” or higher may result in progressive discipline up to and including termination.

5.1.2 Records for relevant qualifications, training, skills, and experience of technical personnel 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.

5.1.2.1 Technical personnel include those individuals who are involved in testing and support of testing (e.g. making reagents, maintaining instruments, etc.) of forensic casework or database samples. Individuals not involved in the stream of testing (e.g. evidence management, sample control, administrative, clerical) are not considered technical personnel.

5.2 **Technical Manager**

Any individual filling the position of the OSBI Forensic Biology Discipline Technical Manager must meet or exceed the minimum education, experience, training requirements, and qualifications as per the QAS (reference CWQM_4.2). In addition, all requirements of the Criminalist IV classification maintained by the OMES HCM Division of the State of Oklahoma shall also be satisfied. The technical manager shall be a full-time employee of the OSBI CSD and meet the following qualifications:

5.2.1 The minimum educational requirements of the QAS. The technical manager shall have, at a minimum, a master's degree in a biology-, chemistry-, or forensic science-related area and shall have successfully completed twelve cumulative semester hours or equivalent from a combination of undergraduate and graduate coursework covering the following subject areas: biochemistry, genetics, molecular biology, and statistics or population genetics.

5.2.1.1 The twelve semester or equivalent credit hours shall include at least one graduate level course registering three or more semester or equivalent credit hours.

5.2.1.2 The specific subject areas listed in 5.2.1 shall constitute an integral component of any coursework used to demonstrate compliance.

5.2.1.3 Individuals who have completed coursework with titles other than those listed in 5.2.1 shall demonstrate compliance through a combination of pertinent materials, such as a syllabus, letter from the instructor, or other document that supports the course content.

5.2.1.4 If the degree requirements of 5.2.1 were waived by the American Society of Crime Laboratory Directors (ASCLD) in accordance with criteria approved by the FBI Director, such a documented waiver shall be permanent and portable.

5.2.2 The minimum experience requirements of the QAS. Any technical manager appointed on or after July 1, 2009, shall have a minimum of three years of human DNA (current or previous) experience as a qualified analyst on forensic samples.

5.2.3 Any technical manager appointed on or after July 1, 2020 shall be a currently or previously qualified analyst in each technology utilized in the laboratory or have documented training in each technology utilized in the laboratory within one year of appointment.

5.2.4 The technical manager shall have previously completed or will successfully complete the FBI's DNA auditor training course within one year of appointment.

5.2.5 The duties, authority, and responsibilities of the technical manager will be, at a minimum, those designated by the QAS. Additional duties, authorities, and responsibilities can be required of the technical manager, as necessary, to meet the needs of the OSBI. The technical manager shall have the following authority and minimum responsibilities:

5.2.5.1 Oversee the technical operations of the OSBI Forensic Biology multi-laboratory system (discipline), which includes the CODIS and forensic biology units.

5.2.5.2 Authority to initiate, suspend, and resume technical operations for a laboratory or an individual. Note, while other laboratory personnel, such as the OSBI CSD director or quality manager may also have the authority to suspend technical operations for a laboratory or an individual, the authorization of the technical manager is required to initiate or resume the technical operations for a laboratory or an individual.

5.2.5.3 Evaluate and approve all validations and new or modified methods used by the laboratory.

5.2.5.4 Review the training records for newly qualified analysts, technicians and technical reviewers and approve their qualifications prior to independent casework analysis. Review, verify, and approve the academic transcripts for newly qualified analysts and technical reviewers.

5.2.5.5 Approve the technical specifications for outsourcing agreements.

5.2.5.6 Review internal and external DNA Audit documents and, if applicable, approve corrective action(s).

5.2.5.7 Review, on an annual basis, the procedures of the laboratory.

5.2.5.8 Review and approve the training, quality assurance, and proficiency testing programs in the laboratory.

5.2.5.9 Review potential conflicts of interest when contract employees are employed by multiple NDIS participating and/or vendor laboratories.

5.2.6 The technical manager shall be accessible to provide on-site, telephone, or electronic consultation as needed. The OSBI Forensic Biology multi-laboratory system will have one technical manager over the system of separate laboratory facilities. The technical manager shall conduct and document a site visit to all satellite laboratories performing DNA analysis in the OSBI Forensic Biology multi-laboratory system at least semi-annually.

5.2.7 A newly appointed technical manager will be responsible for the documented review of the following within one year of appointment or preferably sooner, if possible:

5.2.7.1 Validation studies and analytical procedures currently used by the laboratory; and

5.2.7.2 Educational and training records of currently qualified analysts and technical reviewers.

5.3 **Casework CODIS Administrator**

Any individual filling the position of the OSBI Casework CODIS administrator must meet or exceed the minimum education, experience, training requirements, and qualifications as per the QAS (reference CWQM_4.2) and the NDIS Operational Procedures Manual. The casework CODIS administrator shall be an employee of the OSBI CSD and meet the following qualifications:

5.3.1 The minimum educational requirements for an analyst as defined by the QAS (reference 5.4 below). A casework CODIS administrator appointed prior to July 1, 2020 shall be deemed to have satisfied the minimum education requirements; satisfaction of these minimum educational requirements shall be applicable to the specific laboratory by which the casework CODIS administrator is employed by prior July 1, 2020 and shall not be portable.

5.3.2 The minimum experience requirements of the QAS. The casework CODIS administrator shall be a current or previously qualified analyst as defined by the QAS (reference 5.4 below) with documented mixture interpretation training.

5.3.3 The minimum CODIS training requirements of the QAS. The casework CODIS administrator shall successfully complete the FBI-sponsored training in CODIS software within six months of assuming CODIS casework administrator duties if not previously completed. The casework CODIS administrator shall successfully complete the FBI's DNA auditor training course within one year of assuming his/her administrator duties if not previously completed.

5.3.4 The duties and responsibilities of the OSBI casework CODIS administrator will be, at a minimum, those designated by the QAS and the NDIS Operational Procedures Manual, and as listed in the OSBI CODIS Unit Policy Manual. Additional duties and responsibilities can be required of the casework CODIS administrator, as necessary, to meet the needs of the OSBI. The casework CODIS administrator shall have the following minimum responsibilities:

5.3.4.1 Administer the laboratory's CODIS network.

5.3.4.2 Schedule and document the CODIS computer training of casework analysts.

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

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

5.3.4.5 Ensure that matches are dispositioned in accordance with NDIS operational procedures.

5.3.5 The casework CODIS administrator, in consultation with the OSBI CSD director and quality manager, shall be authorized to terminate an analyst's or laboratory's participation in CODIS until the reliability and security of the computer data can be assured in the event an issue with the data is identified.

5.3.6 For the OSBI, the casework CODIS administrator position will be filled by the state CODIS administrator. In the event that the casework CODIS administrator position is unoccupied, no DNA profiles will be uploaded to NDIS. However, if the alternate CODIS administrator[2] is available to assume the casework CODIS administrator responsibilities, the laboratory may continue to upload DNA profiles to NDIS.

5.4 **Lead Analyst**

A Senior Criminalist designated to assist the Forensic Biology Discipline Technical Manager in one of the casework or CODIS areas specified below. Lead analysts are expected to spend approximately 25% of their time working on these assignments, and approximately 75% of their time performing casework and/or other assigned duties. Senior Criminalists assigned as Lead Analysts are not required to meet the QAS requirements for a Technical Manager, as the Forensic Biology Technical Manager will retain ultimate responsibility for and authority over the technical operations of the Forensic Biology Units. The Lead Analyst positions may serve as succession planning positions for senior analysts who are interested in and are qualified (or plan to become qualified) to serve in the TM position in the future. Lead analysts must meet the minimum education and experience qualifications of an Analyst, as defined in section 5.5.

5.4.1 Lead Analyst – Training:

- Oversees training of and/or serves as designated trainer for serology, DNA, and technician trainees
- Assists TM in preparation of training and/or competency exercises for newly implemented/ validated analysis methods
- Quarterly literature review: finds articles and sends out list to analysts, maintains review documentation
- Identifies and sends out notification of relevant CE/training opportunities for analysts
- Reviews analyst training documents to verify annual CE requirements are met and documented
- Other training-related tasks as assigned by TM
- If qualified, serve as Acting Technical Manager when designated by the TM

5.4.2 Lead Analyst – Casework:

- Provides technical consultation to analysts for casework questions or issues that do not require TM approval

[2] Designation described in the FBI National DNA Index System (NDIS) Operational Procedures Manual.

- Assists TM in preparation of casework policy and training manual deviations and revisions
- Assists TM in organization of annual review of casefiles
- Assists TM and other members of Bio Management with organization of casework outsourcing projects
- Assists TM with validations and implementation of new methods for casework
- Assists TM with identification and planning of future validation projects for casework
- Assists TM in review of Proficiency Test results
- Assists with Forensic Genetic Genealogy cases as needed
- Other casework-related tasks as assigned by TM
- If qualified, serve as Acting Technical Manager when designated by the TM

5.4.3 Lead Analyst – CODIS:

- Provides technical consultation to analysts for databasing questions or issues that do not require TM approval
- Assists TM in preparation of databasing policy and training manual deviations and revisions
- Assists TM in organization of annual review of databasing records
- Assists TM and other members of Bio Management with organization of databasing outsourcing projects
- Assists TM with validations and implementation of new methods for databasing
- Assists TM with identification and planning of future validation projects for databasing
- Assists TM in review of Proficiency Test results
- Other databasing-related tasks as assigned by TM
- If qualified, serve as Acting Technical Manager when designated by the TM

5.4.5 Lead Analyst – QA/QC:

- Assists TM and other members of Bio Management with internal audits and external assessments
- Works with technicians to organize completion of weekly/monthly/annual QC and maintenance tasks
- Reviews QC records to ensure documentation is correct and complete
- Reviews equipment maintenance records to ensure documentation is correct and complete
- Assists TM with troubleshooting issues with equipment, kits, and reagents
- Other QA/QC-related tasks as assigned by TM
- If qualified, serve as Acting Technical Manager when designated by the TM

Note: per QAS 5.2.5, the ultimate authority and responsibility for the following remains solely on the Technical Manager:

5.2.5.1 Oversee the technical operations of the laboratory.
5.2.5.2 Authority to initiate, suspend, and resume technical operations for the laboratory or an individual.
5.2.5.3 Evaluate and approve all validations and new or modified methods used by the laboratory.
5.2.5.4 Review the training records for newly qualified analysts, technicians and technical reviewers and approve their qualifications prior to independent casework analysis. Review, verify, and approve the academic transcripts for newly qualified analysts and technical reviewers.
5.2.5.5 Approve the technical specifications for outsourcing agreements.
5.2.5.6 Review internal and external DNA Audit documents and, if applicable, approve corrective action(s).
5.2.5.7 Review, on an annual basis, the procedures of the laboratory.

5.2.5.8 Review and approve the training, quality assurance, and proficiency testing programs in the laboratory.
5.2.5.9 Review potential conflicts of interest when contract employees are employed by multiple NDIS participating and/or vendor laboratories.

5.5 **Analyst**

Any individual filling the position of a DNA Analyst (Analyst) must meet or exceed the minimum education and experience requirements as per the QAS (reference CWQM_4.2). An analyst shall be an employee or contract employee of the OSBI CSD and meet the following qualifications:

5.5.1 The minimum educational requirements of the QAS. The analyst shall have a bachelor's (or its equivalent) or an advanced degree in biology-, chemistry-, or forensic science-related area and shall have successfully completed coursework (graduate or undergraduate level) covering the following subject areas: biochemistry, genetics, and molecular biology. Any analyst hired/appointed/promoted (as defined by CWQM_4.2) prior to July 1, 2020, shall have coursework and/or training in statistics and/or population genetics as it applies to forensic DNA analysis. Any analyst hired/appointed/promoted on or after July 1, 2020, shall have successfully completed coursework covering statistics and/or population genetics.

5.5.1.1 The specific subject areas listed in 5.4.1 shall be an integral component of any coursework used to demonstrate compliance.

5.5.1.2 Analysts appointed or hired on or after July 1, 2009 shall have a minimum of nine cumulative semester hours or equivalent that cover the required subject areas of biochemistry, genetics, and molecular biology.

5.5.1.3 Analysts who have completed coursework with titles other than those listed in 5.4.1 shall demonstrate compliance through a combination of pertinent materials, such as a syllabus, letter from the instructor, or other document that supports the course content. The technical manager shall approve compliance.

5.5.2 The minimum experience requirements of the QAS. The analyst shall have six months of documented forensic human DNA laboratory experience before release for independent casework analysis. If prior forensic human DNA laboratory experience is accepted by the laboratory, the prior experience shall be documented and augmented by additional training, as needed. The analyst shall successfully complete the required training.

5.6 **Technical Reviewers**

Any individual filling the position of a Technical Reviewer must meet or exceed the minimum education and experience requirements for an analyst as per the QAS (reference CWQM_4.2) and listed in 5.4 above. A technical reviewer shall be an employee or contract employee of the OSBI CSD and meet the following qualifications:

5.6.1 A current or previously qualified analyst in the methodologies being reviewed.

5.6.2 Successful completion of documented training.

5.7 **Technicians**

Any individual filling the position of a casework Laboratory Analyst (Technician) shall be an employee or contract employee of the OSBI CSD and meet the following qualifications:

5.7.1 Possess the equivalent of an associate's degree with some laboratory experience.

5.7.2 Successful completion of documented training.

5.8 **Laboratory Support Personnel**
Any individual filling the position of Laboratory Support Personnel, such as an administrative technician, shall have documented training specific to their job function(s).

5.9 The technical manager will verify and approve the education, to include a review of academic transcripts, of each analyst and technical reviewer prior to release for independent casework analysis and/or technical review.

Attachment(s):
None

Reference(s):
OSBI Memorandum to the Forensic Biology Discipline: “Selective Qualifications for Forensic Biologist/CODIS Analyst Positions with the OSBI”

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI CODIS Unit Policy Manual

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

FBI National DNA Index System (NDIS) Operational Procedures Manual

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

CWQM_6 TRAINING (↑ Table of Contents)

6.1 **Training Program**

The training program will comply with OSBI CSD QP 19 (Training) and meet the requirements of QAS. The OSBI forensic biology units' training program for qualifying analysts and technicians is documented in the "OSBI Forensic Biology Units' Training Manual." The training manual is designed to allow an analyst or technician to successfully complete documented training and be authorized in specific methods, methodologies, or responsibilities independent of the whole manual, where necessary, prior to participating in independent casework analysis. The training program shall:

6.1.1 Address all DNA analytical, interpretation, and/or statistical procedures used in the laboratory.

6.1.2 Include practical exercises encompassing the examination of a range of samples routinely encountered in casework. Practical exercises are not limited to lab work but can also be in the form of data analysis and review. Examples of a range of samples routinely encountered may include degraded, partial, mixed contributor, low template, off-ladder alleles and microvariant samples.

6.1.3 Teach and assess the technical skills and knowledge required to perform DNA analysis.

6.1.3.1 The training program for analysts shall include the skills and knowledge required to conduct a technical review.

6.1.4 Include an assessment of oral communication skills and/or a mock court exercise. This applies to analysts and technicians.

6.1.5 Include requirements for competency testing (see 6.3 below).

6.2 **Training Program Modifications**

The technical manager shall approve any modifications to an analyst's, technical reviewer's, technician's, or laboratory support personnel's required training based on the documented assessment of the individual's previous training and experience with regards to the adequacy and applicability of it to the procedures of the OSBI forensic biology units.

6.3 **Competency Testing (for Analysts/Technicians Completing the Training Program)**

This section applies to analysts or technicians completing the laboratory's training program who will be authorized to perform independent casework analysis for the first time as an analyst or technician in the laboratory (e.g. a new hire or a technician promoted to analyst).

All analyst/technician(s), regardless of previous experience, shall successfully complete competency testing covering the routine DNA methods, interpretation, and/or statistical procedures that the analyst/technician will perform prior to participating in independent casework. The OSBI forensic biology units consider exams/quizzes, mock cases/samples, mock court exercises and technical question assessments "competency tests." Any practical component of competency testing should be relevant to the task(s) that the trainee will be authorized to perform on casework. A passing grade for all types of competency tests will be 80% unless deemed otherwise and documented by the technical manager.

6.3.1 Competency testing for a new analyst shall include a practical component and written and/or oral components sufficient to demonstrate that the trainee has achieved the technical skills and knowledge necessary to perform and explain forensic DNA analysis.

6.3.2 Competency testing for a new technician shall include a practical component sufficient to demonstrate that the trainee has achieved the technical skills and knowledge necessary to perform the forensic DNA methods.

6.4 **Qualification of Currently or Previously Qualified Analyst/Technician in New or Additional Method**

This section will be applicable when an analyst or technician who has completed the laboratory training program is undergoing training in an additional method for which they are not currently qualified or when an analyst or technician is trained in a newly validated and implemented method.

For an analyst or technician, currently or previously qualified within the laboratory, to be qualified in a new or additional method, the laboratory shall teach and assess the technical skills and knowledge required to perform the additional method. For an analyst who also performs technical review, elements of both roles need to be addressed in the training.

6.4.1 Before the use of a new or additional method on forensic samples or casework reference samples, the analyst and/or technician shall successfully complete competency testing to the extent of his/her participation in casework analyses. The competency testing shall include a practical component.

6.5 **Qualification of Currently or Previously Qualified Analyst to Interpret Data & Generate Reports for New or Additional Technology, Typing Test Kit, Platform, or Interpretation Software**

This section will be applicable when a qualified analyst is trained in the interpretation of data using an additional technology, typing test kit, platform, or interpretation software for which they are not currently qualified or when the laboratory analysts are trained in a newly validated and implemented technology, typing test kit, platform, or interpretation software.

For an analyst, currently or previously qualified within the laboratory, to be qualified to interpret data and generate reports for a new or additional technology, typing test kit, platform, or interpretation software, the laboratory shall teach and assess the technical skills and knowledge required to interpret data, reach conclusions, and generate reports using the additional technology, typing test kit, platform, or interpretation software. For an analyst who also performs technical review, elements of both roles need to be addressed in the training.

The training for interpretation software pertains to the implementation of new or additional software. Updated or modified interpretation software with fundamental changes that requires analysts to learn new skills and knowledge to interpret data, reach conclusions, or generate report would also require training as applicable under this section.

In instances where the technology, typing test kit, platform, or interpretation software also involve training in a new method(s), both section 6.4 (above) and 6.5 apply. In these instances, the competency testing may be combined.

6.5.1 Before the use of a new or additional technology, test typing kit, platform or interpretation software on forensic samples or casework reference samples, the analyst shall successfully complete competency testing using the additional technology, typing test kit, platform or interpretation software to the extent of his/her participation in casework analyses. The competency testing shall include a practical component.

6.6 **Technical Reviewer Not Currently Qualified as an Analyst**

This section applies to individuals who will be trained and authorized to conduct technical reviews but are not or will not be authorized as an analyst in the method, technology, typing test kit, platform, or interpretation software (or legacy version).

A technical reviewer, who is not currently qualified as an analyst in the laboratory, shall receive training on the case notes, data analysis, interpretation, and reporting criteria for any method,

technology, typing test kit, platform, or interpretation software or the legacy technology, typing test kit, platform and/or interpretation software on which they were not previously qualified as an analyst in the laboratory.

6.6.1 The technical reviewer shall successfully complete competency testing before completing a technical review of data and/or reports using the additional method, technology, typing test kit, platform or interpretation software used in casework analysis.

6.6.1.1 For a technical reviewer who is a contract employee conducting reviews for an NDIS participating laboratory, the competency testing shall be administered by the NDIS participating laboratory.

6.7 **Qualification of Analyst Not Previously Qualified for Reinterpretation of Legacy Data**
This section applies to analysts who were not previously qualified in the laboratory to interpret data from a legacy technology, typing test kit, and/or platform and will be authorized to reinterpret legacy data.

For an analyst to be qualified in reinterpretation of legacy data, for which they were not previously qualified within the laboratory, the analyst shall demonstrate the technical skills and knowledge required to interpret data, reach conclusions, and generate reports in the legacy technology, typing test kit, and/or platform. The training should address the laboratory's procedures for the reinterpretation of legacy data (refer to CWQM_9.11).

6.7.1 The analyst shall successfully complete competency testing in the legacy technology, typing test kit, and/or platform to the extent of his/her participation in casework analyses. The competency testing shall include practical components of reinterpretation.

6.8 **Laboratory Procedures for Proficiency of Analyst/Technical Reviewer Reinterpreting Legacy Data**
The laboratory shall have and follow procedures for maintaining or reestablishing the technical skills and knowledge of analysts and technical reviewers who reinterpret legacy data for which they are qualified or previously qualified and whose external proficiency testing does not include a legacy technology, typing test kit or platform.

6.8.1 The technical manager shall review the documentation of an analyst's or technical reviewer's maintenance or reestablishment of the technical skills and knowledge and authorize the analyst or technical reviewer to reinterpret legacy data for no more than a two year period.

6.9 The technical manager shall review the training records for the analyst, technician, and/or technical reviewer and approve his/her qualifications prior to independent casework responsibilities.

6.10 **Authorization**
The analyst, technician, and/or technical reviewer shall be authorized to independently perform assigned job responsibilities and the date(s) shall be documented.

6.10.1 The technical manager will issue a memorandum formally recognizing an individual's successful completion of training and releasing them to perform specific responsibilities. This memorandum will include the scope of the release, clearly stating the approval to conduct independent forensic DNA analysis using the applicable methods, technologies, typing test kits, and platforms and/or to perform technical reviews.

6.10.2 The technical manager will issue an "Authorization to Work" (ATW) memorandum, when necessary, to include testing, instrumentation/equipment, reporting/authorization of results, technical review, testimony (opinions and interpretations), etc. as required for accreditation.

6.10.3 The technical manager may also authorize individuals to perform specific laboratory activities as listed in QP 19 (Training), including, but not limited to, assisting with development, modification, verification, and internal validation of methods. This authorization does not necessarily have to be included in an ATW but rather can be provided via email or a memorandum.

6.11 **Laboratory Support Personnel**

Laboratory support personnel shall have documented training specific to their job function(s). Training should include, at a minimum, those task(s) that are necessary for performance of or may impact the results of an analytical procedure (e.g. making reagents or preparing an instrument for operation).

6.12 **Retraining**

Retraining of an analyst, technician, or technical reviewer may be necessary as a result of an extended absence from casework, as part of corrective action, or when determined necessary by the technical manager. The laboratory shall have and follow a policy for addressing retraining of personnel when necessary. The technical manager shall be responsible for evaluating the need for and addressing the extent of retraining. The retraining plan shall be documented and approved by the technical manager.

6.12.1 The individual shall successfully complete competency testing prior to his/her return to participation in casework analyses. This also applies to individuals who have been on extended leave for a period of time that takes them out of the proficiency test cycle. This competency testing shall include a practical component.

6.13 **Maintaining Records**

Records on the training of the laboratory personnel, including successful completion of competency testing, shall be maintained in each individual's training notebook and/or on the appropriate network server.

6.13.1 The training and authorization records for each analyst, technician, and technical reviewer must be available for review.

6.13.2 The documented training completed by each laboratory support personnel must be available for review.

Attachment(s):
None

Reference(s):
OSBI Forensic Biology Units' Training Manual

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

SWGDAM Training Guidelines

CWQM_7 FACILITIES & EVIDENCE CONTROL (↑ Table of Contents)

7.1 The OSBI forensic biology multi-laboratory system shall have facilities designed to ensure the integrity of the analyses and the evidence.

7.1.1 The OSBI forensic biology units shall have secure, controlled access areas for evidence storage as outlined in OSBI CSD QP 6.1 (Evidence Handling), QP 6.2 (Evidence Handling for Non-Casework Purposes), QP 6.3 (Evidence Storage & Maintenance), and QP 6.4 (Evidence Refrigerator & Freezer Maintenance).

7.1.2 Except as provided in 7.1.3.1, techniques performed prior to PCR amplification such as evidence examinations, DNA extractions, and PCR setup shall be conducted at separate times or in separate physical spaces from each other.

7.1.3 Except as provided in 7.1.3.1, amplified DNA product, including real time PCR, shall be generated, processed, and maintained in a room(s) separate from the evidence examination, DNA extractions, and PCR setup areas. The door(s) between rooms containing amplified DNA and other areas shall remain closed except for passage.

7.1.3.1 If applicable, a Rapid DNA instrument//System used for processing casework reference samples shall be maintained in rooms outside of evidence examination areas or those containing amplified DNA.

7.2 The OSBI forensic biology units will follow the procedures in OSBI CSD QP 20 (Lab Security) for laboratory security.

7.2.1 Access to the laboratory shall be controlled and limited in a manner to prevent access to the operational areas by unauthorized personnel. All exterior entrance/exit points require security controls that limit entry and access into the operational areas. The distribution of all keys, combinations, etc., shall be documented and limited to the personnel designated by laboratory management.

7.3 To ensure the integrity of physical evidence, the OSBI forensic biology units follow a documented evidence control program as addressed in 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 & Maintenance), QP 6.4 (Evidence Refrigerator & Freezer Maintenance), QP 7 (Evidence Transactions), and QP 20 (Lab Security).

7.3.1 Evidence shall be marked with case number and item number (unique identifier) on the evidence packaging in accordance with the OSBI CSD Quality Procedures.

Evidence is defined in the OSBI CSD Quality Manual. Work product is defined in CWQM_2 (Definitions), 7.3.3 below, and CASE_8 (Retention of Samples).

Evidence shall be clearly distinguishable from all other evidence at all times throughout processing. Case number and item number shall be present on the evidence in accordance with the OSBI CSD Quality Procedures. When batching is used, case numbers and item numbers shall be present on all work products, when possible, or they shall otherwise be designated in a manner to clearly distinguish each sample throughout processing (e.g. plate map).

7.3.2 Chain of custody for all evidence 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 & Maintenance), QP 6.4 (Evidence Refrigerator & Freezer Maintenance), and QP 7 (Evidence Transactions).

7.3.3 Evidence and work product 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 & Maintenance), and QP 6.4 (Evidence Refrigerator & Freezer Maintenance) in order to minimize loss, contamination, and/or deleterious change and to preserve their integrity. Work product will also be handled according to the appropriate sections below:

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

Serology Work Product 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 CASE_8 (Retention of Samples).

DNA Work Product Utilized in Testing
Swabs, cuttings and portions of biological samples are considered work products utilizing in testing. Following the completion and documentation of the test results, these work products shall be handled according to CASE_8 (Retention of Samples).

DNA Work Product Generated as a Function of the Analysis Process
DNA extracts, including crude Y-screen lysis extracts; dilutions, including normalized dilutions; and amplified DNA products (PCR tubes/plates 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/test results, extracts and dilutions shall be handled according to CASE_8 (Retention of Samples). Following the completion and documentation of the technical review, all amplified DNA products shall be discarded.

7.3.3.1 Evidence and work product in progress will be secured in accordance with OSBI CSD QP 6.1 (Evidence Handling), QP 6.2 (Evidence Handling for Non-Casework Purposes), QP 6.3 (Evidence Storage & Maintenance), and QP 6.4 (Evidence Refrigerator & Freezer Maintenance).

7.3.3.2 Evidence will be properly sealed in accordance with the OSBI CSD Quality Manual, QMA 3, and Quality Procedures QP 5 (Evidence Intake), QP 6.1 (Evidence Handling), QP 6.3 (Evidence Storage & Maintenance), and QP 7 (Evidence Transactions).

7.4 The OSBI forensic biology casework unit 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 (Notes), CASE_2 (Digital Photography), and CASE_8 (Retention of Samples), for the handling of samples requiring consumption.

7.4.1 Where possible, the laboratory shall retain or return a portion of the evidence sample or extract in accordance with OSBI CSD QP 6.1 (Evidence Handling), QP 6.2 (Evidence Handling for Non-Casework Purposes), QP 6.3 (Evidence Storage & Maintenance), QP 6.4 (Evidence Refrigerator & Freezer Maintenance), QP 16.2 (Contents of Case Records), CASE_8 (Retention of Samples), and as guided by Oklahoma Statutes (Title 22 §1372).

7.5 In accordance with OSBI CSD QP 28 (Report Writing), QP 31 (Reviews), and QMA 1.1, as well as the requirements in CWQM_11 (Reports), CWQM_12 (Review), and CASE_7 (Report Writing), the

OSBI forensic biology units shall report the disposition of evidence for all evidence submitted for analysis to include whether the evidence is returned to the submitting agency, retained by the laboratory, consumed, transferred between OSBI CSD Units, and/or other wording to convey the status of the evidence at the time of reporting the DNA results.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_8 VALIDATION[3] (↑ Table of Contents)

8.1 The OSBI Forensic Biology Discipline shall only use validated methods for DNA analyses.

8.1.1 All validations will be performed in accordance with the requirements of QAS and OSBI CSD QP 21.2 (Evaluation of Methods, Instruments, Equipment, and Software). The type of validation – developmental or internal – will be defined in the validation plan.

8.1.1.1 A developmental validation will follow 8.2.

8.1.1.2 An internal validation will follow 8.3.

8.1.1.2.1 Prior to beginning an internal validation, and if relying upon an externally performed developmental validation, the citations and publications addressing the elements of 8.2.1 must be available and accessible to support the underlying scientific basis.

8.1.1.2.2 The general order of events when conducting an internal validation in the OSBI forensic biology discipline, as per QAS and OSBI CSD QP 21.2 (Evaluation of Methods, Instruments, Equipment, and Software), is as follows:

1. Submit evaluation plan per OSBI CSD QP 21.2.
2. Perform system and site-specific validation studies.
3. Submit documentation and summary for approval.
4. Complete individual analyst/technician competencies per CWQM_6 (Training).
5. Release validated procedure for casework use.

8.1.1.2.3 Individuals assisting with internal validations will be authorized by the technical manager (reference 6.10.3).

8.1.1.3 If a validation apples to serology only, it will be performed in accordance with OSBI CSD QP 21.1 (Evaluation of Methods, Instruments, Equipment, and Software).

8.2 **Developmental Validation**

Developmental validation shall precede the implementation of any new methods used for forensic DNA analysis. The developmental validation documentation may be obtained from an external agency (either commercial vendor or another laboratory) and established through citation and publications as referenced in 8.1.1.2.1.

8.2.1 Developmental validation studies shall include, where applicable, characterization of the genetic marker, species specificity, sensitivity studies, stability studies, case-type samples, population studies, mixture studies, precision and accuracy studies, and PCR-based studies. PCR-based studies include reaction conditions, assessment of differential and preferential amplification, effects of multiplexing, assessment of appropriate controls, and product detection studies. All validation studies shall be documented.

8.2.2 Peer-reviewed publication of the underlying scientific principle(s) of a method shall be required.

[3] Reference CWQM_10 (Equipment) for when performance checks are required, such as for additional critical instruments or equipment of the same model already validated for use in the laboratory.

8.3 **Internal Validation**

Except as provided in 8.3.1.1, internal validation of all manual and robotic methods shall be conducted by each laboratory with the appropriate sample number and type to demonstrate the reliability and potential limitations of the method.

8.3.1 Internal validation studies shall include as applicable: known and non-probative evidence samples or mock evidence samples, precision and accuracy studies, sensitivity and stochastic studies, mixture studies, and contamination assessment studies (reference 8.4 below as well). Studies determined to be not applicable shall be addressed in the internal validation summaries (refer to 8.3.4). If conducted within the same laboratory, developmental validation studies may satisfy some of the elements of the internal validation.

8.3.1.1 Internal validation data may be shared by all locations in the OSBI forensic biology multi-laboratory system. The summary of the shared validation data, including summaries, shall be available at each site and maintained electronically on a secure network server accessible by all laboratories. Each laboratory in the system shall complete, document and maintain their own applicable site-specific precision, sensitivity, and contamination assessment studies. The laboratory responsible for the system internal validation shall incorporate their site-specific studies, as applicable, into the system study.

8.3.2 Internal validation shall define quality assurance parameters and interpretation guidelines, including, as applicable, guidelines for mixture interpretation and the application of appropriate statistical calculations and incorporate such into applicable analytical procedure(s).

8.3.2.1 Mixture interpretation validation studies shall include samples with a range of the number of contributors, template amounts, and mixture ratios expected to be interpreted in casework.

8.3.3 Internal validation studies shall be conducted prior to implementing a change in typing test kit or platform instrument model (e.g. to a new capillary electrophoresis instrument model not already used in the laboratory).

8.3.4 Internal validation studies shall be documented and summarized. Internal validation shall be reviewed and approved by the technical manager prior to implementing a procedure for forensic applications.

8.4 **NIST-Traceable Testing**

Newly validated DNA methods (from amplification through characterization), typing test kit, or platform instrument model shall be checked against an appropriate and available certified reference material (or sample made traceable to the certified reference material) prior to the implementation of the method for forensic analysis.

8.5 **Modified Procedure Evaluations**[4]

The performance of a modified procedure (alteration to validated steps, reagents, or critical instruments that would require a protocol change) shall be evaluated by comparison to the original procedure using similar DNA samples and the evaluation documented. The evaluation shall be reviewed and approved by the technical manager prior to the implementation of the modified procedure into casework applications. Note, modifications that impact efficacy or reliability of the analysis may require internal validation studies as well to demonstrate the continued reliability and potential limitations of the method. Modifications to procedures will be handled in accordance with OSBI CSD QP 21.1 (Research) and/or OSBI CSD QP 21.2 (Evaluation of Methods, Instruments, Equipment, and Software). Approval shall occur through the issuance of a deviation in accordance

[4] Modifications that do not affect the analytical process only require a performance check.

with OSBI CSD QP 3 (Deviations) or a new protocol revision in accordance with OSBI CSD QP 2 (Document Control).

8.6 If applicable, a Rapid DNA instrument used for modified Rapid DNA analysis on casework reference samples shall be validated in accordance with QAS.

8.7 If applicable, an NDIS approved Rapid DNA System shall require a performance check prior to use on casework reference samples.

8.8 **Software Testing**
New software or new modules of existing software and modifications to software will first be evaluated to assess the suitability of the software for its intended use in the laboratory and to determine the necessity of validation studies or software testing. For the purposes of this policy, software includes software tool(s) developed by the laboratory.[5] This evaluation shall include the determination of which studies will and will not be conducted and shall be documented.

8.8.1 **Software Developmental Validation**
New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations, shall be subject to developmental validation prior to implementation in forensic DNA analysis.

8.8.1.1 With the exception of legally protected information, the underlying scientific principle(s) utilized by software with an impact on the analytical process, interpretation, or statistical calculations shall be publicly available for review or published in a peer-reviewed scientific journal.

8.8.1.2 Developmental software validation studies for new software or new modules of existing software used as a component of instrumentation shall include a minimum, functional testing and reliability testing.

8.8.1.3 Developmental software validation studies for new software or new modules of existing software for the analysis and/or interpretation of DNA data shall include at a minimum, functional testing, reliability testing, and as applicable, accuracy, precision, sensitivity, and specificity studies.

8.8.1.4 Developmental software validation studies for new software or new modules of existing software for statistical calculations shall include at a minimum, functional testing, reliability testing, and as applicable, accuracy, and precision studies.

8.8.2 **Software Internal Validation**
New software or new modules of existing software that are used as a component of instrumentation, for the analysis and/or interpretation of DNA data, or for statistical calculations shall be subject to internal validation specific to the laboratory's intended use prior to implementation in forensic DNA analysis.

8.8.2.1 Internal software validation studies for new software or new modules of existing software used as a component of instrumentation shall include functional testing and reliability testing.

[5] For commercial off-the-shelf (COTS) software products (e.g. word processing, electronic spreadsheets, database management) that the laboratory uses to create software tools (e.g. macros, workbooks, databases), the COTS software does not require a developmental validation but the laboratory developed tool must be validated as appropriate for its intended use in the laboratory.

8.8.2.2 Internal software validation studies for new software or new modules of existing software for the analysis and/or interpretation of DNA data shall include functional testing, reliability testing, and, as applicable, precision and accuracy studies, sensitivity, and specificity studies.

8.8.2.3 Internal software validation studies for new software or new modules of existing software for statistical calculations shall include functional testing, reliability testing, and, as applicable, precision and accuracy studies.

8.8.2.4 Software that does not impact the analytical process, interpretation, or statistical calculations (e.g. LIMS or other inventory/sample tracking software) shall require at a minimum, a functional test.

8.8.3 **Software Modifications**
Modifications to software as described in 8.8.1 and 8.8.2 shall be evaluated to determine if the modifications result in major or minor revisions to the software. Release notes from software developers can be used to assist in determining if a modification results in a major or minor revision. Examples of major revisions include, but are not limited to, changes to any algorithm, any statistical and/or calculation equation, sequence alignment strategy, data reports, and/or export of results. Examples of minor revisions include, but are not limited to, cosmetic modifications, improved printing or viewing features, and/or fixing invalid error messages.

8.8.3.1 A major revision to software used as a component of instrumentation shall require validation prior to implementation. Software validation studies shall include functional testing, reliability testing, and regression testing.

8.8.3.2 A major revision to software used for the analysis and/or interpretation of DNA data shall require validation prior to implementation. Software validation studies shall include functional resting, reliability testing, regression testing, and, as applicable, precision and accuracy studies, sensitivity, and specificity studies.

8.8.3.3 A major revision to software used for statistical calculations shall require validation prior to implementation. Software validation studies shall include functional testing, reliability testing, regression testing, and, as applicable, precision and accuracy studies.

8.8.3.4 A minor revision to software that does not impact the analytical process, interpretation, or statistical calculations shall require at a minimum, a functional test.

8.8.4 Software validation studies and software testing may be shared by all locations in the OSBI forensic biology multi-laboratory system. The summary of the shared validation data shall be available at each site and maintained electronically on a secure network server accessible by all laboratories. Each laboratory in the system shall complete, document and maintain their own applicable site-specific reliability testing. The laboratory responsible for the system software validation studies and software testing shall incorporate their site-specific reliability testing into the system study.

8.8.5 Software validation and testing shall be documented. Software validation and testing shall be reviewed and approved by the technical manager prior to implementation.

8.9 Developmental validation studies, internal validation studies, modified procedure evaluations, and software testing, including the approval of the technical manager, shall be retained and available for review.

Quality Manual

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

SWGDAM Validation Guidelines for DNA Analysis Methods

CWQM_9 ANALYTICAL PROCEDURES (↑ Table of Contents)

9.1 The OSBI forensic biology units shall follow analytical procedures supported by the internal validations and approved by the technical manager, in addition to any other corresponding documents referenced within this policy manual, as applicable.

9.1.1 The laboratory will follow the standard operating procedures outlined in this policy manual for each analytical method used, including the appropriate analytical controls required for DNA analysis and data interpretation. All procedures shall be current, readily available, adhere to OSBI CSD QP 2 (Document Control), and contain the following, as applicable:

- Scope
- Reagents & Supplies
- Equipment
- Sample/Reagent Preparation
- Extraction Method
- Controls
- Individual Steps of the Procedure
- Interpretation of Results
- Notes

9.2 **Reagents**

The OSBI forensic biology units shall use reagents that are suitable for the methods employed.

9.2.1 The OSBI forensic biology units shall follow OSBI CSD QP 8.1 (Ordering, Receiving, and Verifying Reagents and Supplies) and the applicable procedures within this policy manual for documenting commercial reagents and for the formulation of in-house reagents.

9.2.1.1 The following will be documented on the appropriate quality control reagent (QCR) OSBI Chemical Receipt/Function Verification Worksheet for commercial reagents:

- Receipt Information
- Storage
- Function Verification
- Unsatisfactory Reagent Performance
- Expiration – The 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.

9.2.1.2 The following will be documented on the appropriate QCR OSBI Chemical Formulation/Function Verification Worksheet for in-house reagents:

- Chemical Formulation & Preparation – The amounts of chemicals necessary to prepare each reagent are provided for a standard formulation; however, the final amount of the chemicals used may be changed as long as the proportions of each remain unaltered.
- Storage
- Function Verification
- Unsatisfactory Reagent Performance
- Expiration – If any ingredient of the reagent has an expiration date earlier than that of the prepared reagent, the expiration date shall be determined by such ingredient with the earliest expiration

9.2.1.3 The technical manager and/or lead analyst will be notified if changes have been made to the product insert for any reagent.

9.2.1.4 Items that are too small to legibly record all required information are exempt from the labeling and date opened requirements as long as the storage container is labeled with all of the appropriate information. (This generally only applies to tubes from kits.)

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

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

9.2.2 Commercial reagents shall be labeled with the identity of the reagent and the expiration date as provided by the manufacturer or as otherwise determined by the laboratory, if applicable, in addition to the requirements of OSBI CSD QP 8.1 (Ordering, Receiving, and Verifying Reagents and Supplies).

9.2.3 In-house reagents shall be labeled with the identity of the reagent, the date of preparation (lot number) and expiration, if applicable, and the identity of the individual preparing the reagent.

9.3 **Critical Reagents**[6]

The OSBI forensic biology units identify the following reagents used in forensic DNA analysis as critical reagents and shall evaluate them prior to use in casework:

9.3.1 Test kits or systems for DNA quantification or amplification (including master mix, primer sets, and allelic ladders, where applicable):

- Quantifiler™ Trio DNA Quantification Kit
- GlobalFiler™ PCR Amplification Kit
- Yfiler™ Plus PCR Amplification Kit

9.3.2 Thermostable DNA polymerase, primer sets and allelic ladders used for genetic analysis that are not tested as test kit components under 9.3.1, if applicable.

9.3.3 Rapid DNA cartridges, if applicable.

9.3.4 Other laboratory defined critical reagents:

- TE^{-4}
- Proteinase K
- Dithiothreitol (DTT)
- PrepFiler *Express*™ and PrepFiler *Express* BTA™ Forensic DNA Extraction Kits
- Differential extraction reagents (for PrepFiler extractions on the AutoMate *Express*™ Forensic DNA Extraction System)

9.4 Except as provided in 9.4.1, the OSBI forensic biology units shall quantify or otherwise calculate the amount of human and/or human male DNA in all forensic samples prior to nuclear DNA amplification.

9.4.1 If applicable, quantification of human DNA for casework reference samples shall not be required if the laboratory has a validated system (e.g. direct amplification and/or Rapid DNA instruments/Systems) demonstrated to reliability yield successful DNA amplification and typing without prior quantification.

[6] No reagents used in serological analysis shall be considered as critical reagents.

9.5 **Monitoring Analytical Procedures Using Controls & Standards**
Except for Rapid DNA instruments/Systems used to analyze casework reference samples pursuant to 9.7 and/or 9.8 (if applicable), the OSBI forensic biology units shall, at minimum, monitor analytical procedures using the following analytical controls and standards. Additional criteria for monitoring analytical procedures, including defining acceptable results for standards and controls and documenting they have been verified, are specified in the applicable procedures in this policy manual.

9.5.1 Reagent blank controls associated with each extraction set being analyzed shall be:

9.5.1.1 Extracted concurrently and treated with the most sensitive conditions as the samples;

9.5.1.2 Amplified utilizing the same typing test kit, instrument model, and sensitivity conditions as required by the sample(s) containing the least amount of DNA; and

9.5.1.3 Typed utilizing the same instrument model, injection conditions and most sensitive volume conditions of the extraction set.

9.5.2 Where quantification is used, quantification standards shall be used. If a virtual or external standard curve is utilized, a calibrator must be ran concurrently with the samples.

9.5.3 Positive and negative amplification controls associated with samples being typed shall be amplified concurrently using the same typing test kit on the same instrument as the samples.

9.5.3.1 Except as provided in 9.5.4.1, all samples typed shall also have the corresponding amplification controls typed.

9.5.4 If applicable, laboratories performing sequencing shall use positive and negative sequencing controls concurrently sequenced using the same typing test kit on the same instrument as the samples.

9.5.4.1 If the positive amplification control is not used as the positive sequencing control, the laboratory shall have and follow procedures for the evaluation of the positive amplification control.

9.5.5 Allelic ladders and internal size standards for PCR-based systems, as applicable.

9.6 The OSBI forensic biology units shall follow written guidelines for the interpretation of data that are based on and supported by internal validation studies. As specified in the applicable procedures in this policy manual including, but not limited to, DNA_4 (DNA Quantification) and CASE_5 (DNA Interpretation & Comparison Guidelines), the forensic biology units shall:

9.6.1 Have criteria to evaluate quantification standards, internal size standards, allelic ladders and analytical controls.

9.6.2 Have criteria for the interpretation of non-allelic peaks/signal.

9.6.3 Have criteria for the interpretation of allelic peaks/signal.

9.6.4 Define the thresholds used for interpretation. As appropriate to the interpretation model utilized (i.e. binary), the following thresholds shall be established:

9.6.4.1 Analytical Threshold

9.6.4.2 Stochastic Threshold

9.6.5 Define criteria for uninterpretable data.

9.6.6 Have and follow procedures for mixture interpretation that address the following:

9.6.6.1 The assessment of the number of contributors.

9.6.6.2 The separation of contributors (e.g. major versus minor).

9.6.6.3 The criteria for deducing potential contributors.

9.7 If applicable, for modified Rapid DNA analysis, a laboratory shall:

9.7.1 Have and follow written guidelines for the manual interpretation of data.

9.7.1.1 The laboratory shall verify that the internal size standard and allelic ladder results meet the laboratory's interpretation guidelines.

9.7.2 Have and follow procedures to address the use of positive sample controls and negative sample controls.

9.8 If applicable, for Rapid DNA analysis, a laboratory shall have and follow procedures to address the use of positive sample controls and negative sample controls.

9.8.1 The Rapid DNA cartridge shall include an internal size standard with each sample.

9.9 CASE_5 (DNA Interpretation & Comparison Guidelines) defines criteria for the formulation of inclusionary, exclusionary, and inconclusive conclusions.

9.10 CASE_6 (DNA Statistics) and CASE_7 (Report Writing) outline procedures for statistical calculations and the reporting of results and conclusions and address the following:

9.10.1 The assumptions that can be made when formulating conclusions.

9.10.2 Performing statistical analysis in support of any inclusion that is determined to be relevant in the context of the case.

9.10.3 Documenting of the genetic loci and assumptions used for statistical calculations, at a minimum, in the case notes.

9.10.4 Not using uninterpretable data in statistical calculations.

9.10.5 The approaches to performing statistical calculations.

9.10.5.1 For autosomal STR typing, the procedure shall address homozygous and heterozygous typing results, multiple locus profiles, mixtures, minimum allele frequencies, and where appropriate, biological relationships.

9.10.5.2 For lineage marker testing, the procedure shall address parameters specific for the applicable lineage marker statistical calculations.

9.10.5.3 The laboratory shall use loci that are shown to be in Hardy-Weinberg equilibrium and statistically unlinked, when using the product rule for statistical calculations.

9.10.6 The source of the population database(s) used in any statistical calculation.

9.10.7 The criteria for source attribution declarations, when applicable.

9.11 The OSBI forensic biology units shall follow the procedure in CASE_5 (DNA Interpretation & Comparison Guidelines) to address the reinterpretation of legacy data.

9.12 The OSBI forensic biology units shall follow all applicable procedures in this policy manual for the detection and control of contamination including, but not limited to, CASE_4 (DNA Analysis).

9.12.1 The forensic biology units shall follow QC_1 (Cleaning, Decontamination & Sterilization) for cleaning and decontaminating facilities and equipment.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_Hemastix**

OSBI Forensic Biology Units' Policy Manual **QCR_SER_Takayama**

OSBI Forensic Biology Units' Policy Manual **QCR_SER_RSID-Blood**

OSBI Forensic Biology Units' Policy Manual **QCR_SER_APSpot_Test**

OSBI Forensic Biology Units' Policy Manual **QCR_SER_Seratec**

OSBI Forensic Biology Units' Policy Manual **QCR_SER_Xmas_Tree_Stain**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_YS_NaOH**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_YS_C2-H4-O2**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_PrepFiler_Kits**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_ProK_DTT_Ext_Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_E-Cell_Digest_Buffer**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Autoclaved_Water**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_QF_Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Quant_Standards**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Amp_Kit**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Formamide**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_10 EQUIPMENT (↑ Table of Contents)

10.1 The OSBI forensic biology units shall use equipment suitable for methods employed.

10.1.1 The OSBI forensic biology units shall follow OSBI CSD QP 24 (Calibration and Handling of Equipment) and the applicable procedures outlined in this policy manual.

10.1.2 Laboratory equipment and instruments shall be used as recommended by the manufacturer or as specified in the applicable analytical protocols.

10.1.3 If laboratory equipment or instruments do not function as expected, OSBI CSD QP 24 (Calibration and Handling of Equipment) shall be followed, and the technical manager, lead analyst, unit supervisor or designee will arrange for service or replacement.

10.1.3.1 In the event that a laboratory does not have suitable equipment for the methods in use, the technical manager or designee (e.g. OSBI CSD director, quality manager, etc.) will suspend technical operations, in accordance with CWQM_5.2.5.2 and utilizing OSBI CSD QP 13 (Nonconforming Work), if applicable, until the laboratory re-establishes the suitability of the equipment.

10.2 **Critical Equipment/Instruments**

The OSBI forensic biology units shall identify critical equipment or instruments and follow a program of quality control procedures, as outlined in this policy manual, to ensure all critical equipment and instruments are maintained.

10.2.1 At minimum, the OSBI forensic biology units identify the following as critical:

10.2.1.1 Handheld mechanical pipettes

10.2.1.2 A thermometer traceable to national or international standard(s) (includes any thermometer used for conducting performance checks)

10.2.1.3 Incubators/heat blocks used in analytical procedures

10.2.1.4 Robotic systems

10.2.1.5 Thermal cyclers, including quantitative PCR

10.2.1.6 Thermal cycler temperature verification systems (TVS)

10.2.1.7 Electrophoresis detection systems, including Genetic Analyzers

10.2.1.8 Rapid DNA instruments/Systems, if applicable

10.2.1.9 Any additional instruments or equipment that produce DNA typing results, if applicable

10.3 **Performance Checks**

The OSBI forensic biology units shall have procedures for conducting performance checks of critical equipment or instruments, as outlined in this policy manual, including evaluating results (to include acceptable ranges), addressing unacceptable data, and documenting the completion and subsequent

approval/rejection of the performance check. Calibration may be utilized to performance check equipment as well.[7]

10.3.1 New critical equipment or instruments, not requiring validation, shall undergo a performance check before use in casework analysis. Each additional critical instrument, of the same instrument model validated for use in the laboratory, shall require a performance check prior to use in casework analysis. This initial performance check may be used to demonstrate compliance with 10.3.2 for the current calendar year.

10.3.2 The following critical equipment or instruments shall require annual performance checks:

10.3.2.1 Handheld mechanical pipettes

10.3.2.2 Incubators/heat blocks used in an analytical procedure

10.3.2.3 Robotic systems

10.3.2.4 Thermal cyclers, including quantitative-PCR

10.3.2.5 Thermal cycler temperature verification systems (TVS)

10.3.2.6 Electrophoresis detection systems, including Genetic Analyzers

10.3.2.7 Any additional instruments or equipment that produce DNA typing results, if applicable

10.3.2.8 Other critical equipment or instruments defined by laboratory, if applicable

10.3.3 The following critical equipment or instruments shall require a performance check after repair or service before returning to use in casework analysis:

10.3.3.1 Robotic systems

10.3.3.2 Thermal cyclers, including quantitative-PCR

10.3.3.3 Electrophoresis detection systems, including Genetic Analyzers

10.3.3.4 Rapid DNA instruments/Systems, if applicable

10.3.3.5 Any additional instruments or equipment that produce DNA typing results, if applicable

10.3.3.6 Other laboratory defined critical equipment or instruments, if applicable

10.3.4 If applicable, a Rapid DNA instrument/System shall require a performance check upon installation.

10.3.5 If applicable, a Rapid DNA instrument/System shall undergo a performance check if the Rapid DNA instrument remains idle longer than the period recommended by the instrument specifications or as established by the laboratory.

[7] If performed in accordance with the applicable procedures of this policy manual and meeting the requirements of the OSBI CSD Quality Manual, including, but not limited to, OSBI CSD QM 6.4 (Equipment), OSBI CSD QM 6.5 (Metrological Traceability), OSBI CSD QM 6.6 (Externally Provided Products & Services), OSBI CSD QP 9 (Evaluation of Suppliers), OSBI CSD QP 23 (Measurement Traceability), and OSBI CSD QP 24 (Calibration and Handling of Equipment).

10.4 **Documentation**

The OSBI forensic biology units shall maintain documentation of maintenance, service, repair, and performance checks.

10.4.1 The schedule and maintenance program for equipment and instruments shall follow the requirements described herein CWQM_10 (Equipment) and the applicable quality control procedures outlined in this policy manual.

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

10.4.3 All performance checks must be documented.

10.4.4 The records associated with equipment or instruments should be portable in the event of equipment or instrument transfers within the OSBI forensic biology multi-laboratory system.

10.4.5 If equipment or instrument is permanently removed from service, the associated records should be archived and accessible for historical purposes.

10.4.6 Each piece of equipment or instrument shall be marked to allow the user to readily identify the date of the last performance check as well as the due date for the next required performance check.

Attachment(s):

OSBI Forensic Biology Units' Policy Manual **QC_Decontamination_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Equip_Maint_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Temp_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Compiled_Temp_Logs_&_Safety_QC**

OSBI Forensic Biology Units' Policy Manual **QC_Pipet_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Pipet_PC_Reproducibility_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Thermometer_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Thermomixer_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_ProFlex_Maint_Log**

OSBI Forensic Biology Units' Policy Manual **QC_ProFlex_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_3500_Maint_&_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_AutoMate_Maint_Log**

OSBI Forensic Biology Units' Policy Manual **QC_QS5_Maint_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Centrifuge_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_pH_Meter_Maint_&_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Water_System_Maint_Log**

Quality Manual

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_11 REPORTS (↑ Table of Contents)

11.1 The OSBI forensic biology units shall follow OSBI CSD QP 16.2 (Contents of Case Records) and QP 28 (Report Writing), in addition to the applicable procedures in this policy manual, for taking and maintaining casework notes to support the conclusions drawn in laboratory reports, maintaining all analytical documentation generated by technicians and/or analysts related to case analyses, and retaining, in written, printed, or electronic format, sufficient documentation for each technical analysis to support the report conclusions such that another qualified individual can evaluate what was done and interpret the data.

11.1.1 The OSBI forensic biology units' "Abbreviation List" shall be maintained in accordance with the OSBI CSD Quality Manual.

11.2 Casework reports[8] shall include the following elements and comply with OSBI CSD QP 28 (Report Writing):

11.2.1 Case identifier;

11.2.2 Description of evidence examined and identification of samples tested;

11.2.2.1 Any item(s) collected or created and preserved for future testing from an item of evidence, when applicable, must be included in the report.

11.2.2.2 Any stain, sample, or item on which an attempt is made to isolate DNA, regardless of the outcome or result, must be addressed in the report.

11.2.3 Technology used;

11.2.4 Loci, sequence region, or amplification system;

11.2.5 Results and/or conclusions for each forensic sample tested;

11.2.6 A quantitative or qualitative interpretative statement to support all inclusions;

11.2.7 Date of the report;

11.2.8 Disposition of evidence;

11.2.8.1 Disposition should be specific to the evidence in the report and may include whether the evidence is returned to the submitting agency, retained by the laboratory, consumed, and/or other wording to convey the status of the evidence at the time of reporting the DNA results.

11.2.8.2 The disposition may be a general statement for all items with the same disposition but must convey the status of each of the items of evidence.

11.2.9 A signature and title, or equivalent identification, of the person accepting responsibility for the content of the report.

11.2.9.1 Only one analyst shall accept responsibility for the content of the report. This shall be documented by the Laboratory Information Management System (LIMS)

[8] Casework reports as applicable to DNA analysis. Serology casework reports shall include, at minimum, the elements required by OSBI CSD QP 28 (Report Writing).

secure electronic signature of the analyst on the report in accordance with OSBI CSD QP 28 (Report Writing).

11.3 Except as otherwise provided by state or federal law, reports, case files, DNA records, and databases shall be confidential.[9] The OSBI forensic biology units shall follow OSBI CSD QP 16.1 (Control of Records) and QP 33 (Release of Case Information) to:

11.3.1 Ensure the privacy of the reports, case files, DNA records, and databases;

11.3.2 Release reports, case files, DNA records, and databases, in accordance with applicable state or federal law; and

11.3.3 Release personally identifiable information in accordance with applicable state and federal law.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **CWQM_Abbreviation_List**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

[9] DNA profiles will not be transmitted outside the OSBI network electronically. The OSBI network is secure (internal email and servers); therefore, NDIS allows these electronic transmissions. However, DNA profiles will not be emailed outside of the OSBI network without prior authorization from the Technical Manager.

CWQM_12 REVIEW[10] (↑ Table of Contents)

12.1 The OSBI forensic biology units shall follow the procedure in OSBI CSD QP 31 (Reviews) to conduct and document technical and administrative reviews of all serology and DNA case files and reports prior to releasing official test results to ensure conclusions and supporting data are reasonable and within the constraints of scientific knowledge. Both the administrative and technical review of all case files and reports must be conducted and documented prior to issuing the report. All analysts in the OSBI forensic biology units will route their technical reviews to an assortment of qualified analysts/technical reviewers in a rotation that ensures all available individuals are reviewing one another's casework.

12.1.1 An individual conducting technical reviews shall meet the requirements described in OSBI CSD QP 31 (Reviews); shall be an analyst or technical reviewer qualified in the method, technology, typing test kit, platform, and interpretation software being reviewed; and shall participate in semi-annual external proficiency testing. The individual conducting the technical review shall not be the original (examining) analyst and must be authorized (released) by the technical manager to perform technical reviews.

12.1.1.1 Individuals whose sole responsibility is technical review shall be proficiency tested in the technical review of each applicable technology and typing test kit in accordance with CWQM_13 (Proficiency Testing).

12.1.2 Preliminary test results may be provided to customers prior to technical review, if necessary, in accordance with OSBI CSD QM 7.8.7. When preliminary results are communicated by dialogue to the customer, it must be clearly conveyed to the customer that the results are preliminary (unofficial) and pending technical review, after which, the final, official results will be released via a Criminalistics Examination Report. A record of the dialogue shall be maintained in the case record, including but not limited to the preliminary results provided and to whom.

12.2 Completion of the technical review shall be documented (i.e. completion of a technical review form) in accordance with OSBI CSD QP 31 (Reviews) and the technical review of forensic casework shall include the following elements, at a minimum:

12.2.1 A review of all case notes, all worksheets, and the electronic data (or printouts of such data) supporting the results and/or conclusions.[11]

12.2.2 A review of all analysis parameters, analytical controls, internal size standards, and allelic ladders to verify that the expected results were obtained, except when using an NDIS approved Rapid DNA System on casework reference samples, if applicable.

12.2.3 A review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms or images), except when using an NDIS approved Rapid DNA System on casework reference samples, if applicable.

12.2.4 A review of all data to verify conclusions (i.e. inclusions, exclusions, inconclusive) are in compliance with laboratory guidelines.

12.2.5 A review of statistical analysis, if applicable.

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[10] This applies to data generated within the OSBI forensic biology units. The review of data generated external to the OSBI is governed by CWQM_17 (Outsourcing Ownership).

[11] This includes a review of all calculations, data transfers, etc.; however, a review of such is not required if not subject to human error.

12.2.6 A review of the final report's content to verify compliance with CWQM_11.2 (Reports) and that the results and/or conclusions are supported by the data.

12.2.7 Verification that all profiles entered into CODIS (as documented on the CODIS Data Entry Form[s]) are eligible, have the correct DNA types, and correct specimen category.

12.2.7.1 Prior to upload to SDIS, entry of a DNA profile into a searchable category of SDIS, or search of SDIS, the following criteria will be verified (and documented on a CODIS Data Entry Form) by two concordant assessments by a qualified analyst or technical reviewer: eligibility for CODIS, correct DNA types, and appropriate specimen category.

12.3 Completion of the administrative review shall be documented (i.e. completion of an administrative or technical review form) in accordance with OSBI CSD QP 31 (Reviews) and shall include the following elements, any or all of which may be included within the technical review:

12.3.1 A review of the case file and final report for clerical accuracy and compliance with CWQM_11.2 (Reports).

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

12.4 The OSBI forensic biology units shall follow the procedure in OSBI CSD QP 31 (Reviews) to address unresolved issues and/or unresolved discrepant conclusions between analysts and reviewer(s).

12.5 The OSBI forensic biology units shall follow the procedure(s) in the OSBI CODIS Unit Policy Manual for the verification and resolution of database matches.

Attachment(s):
None

Reference(s):
OSBI Memorandum to the Forensic Biology Discipline: "Analysts Qualified for Technical Review"

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI CODIS Unit Policy Manual

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_13 PROFICIENCY TESTING[12] (↑ Table of Contents)

13.1 Analysts, technical reviewers, technicians, and other personnel designated by the technical manager participating in forensic DNA analysis shall undergo semi-annual[13] external proficiency testing per calendar year; those participating in forensic serology analysis shall undergo external proficiency testing at least once per calendar year. The OSBI forensic biology units shall follow OSBI CSD QM 6.6.2 (Determining Suitability of External Providers) and OSBI CSD QP 30 (Proficiency Tests).

13.1.1 Analysts qualified in more than one DNA technology[14] (e.g. STR, Y-STR, etc.) shall be proficiency tested in each technology at least once per calendar year. All applicable samples in a single proficiency test shall be worked for each technology.

13.1.1.1 Typing of all CODIS core loci or CODIS core sequence ranges shall be attempted for each technology at least once per calendar year.

13.1.2 Analysts qualified in more than one DNA typing test kit (e.g. GlobalFiler™, Yfiler™ Plus, etc.) shall be proficiency tested in each typing test kit at least once per calendar year.

13.1.2.1 If applicable, analysts qualified to perform modified Rapid DNA analysis shall be externally proficiency tested on the interpretation of data generated by each Rapid DNA instrument model for each PCR STR typing test kit at least once per calendar year.

13.1.3 Individuals (analysts, technicians, and other personnel designated by the technical manager) that perform DNA analytical procedures on forensic samples or casework reference samples shall be proficiency tested on at least one method[15] in each methodology[16] for which they are qualified to perform casework analysis at least once per calendar year.

13.1.3.1 For serology proficiency tests, each individual shall complete testing for all serological procedures for which they are authorized, regardless of the scenario provided by the manufacturer.

13.1.4 Except as provided in 13.1.4.1, each external proficiency test shall be assigned to and completed by one analyst.

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[12] The testing of legacy technologies, typing test kits and platforms, if applicable, shall be governed by CWQM_6.8.

[13] *Semi-annual* requires testing to take place two times during one calendar year, with the first event taking place in the first six months of that year and the second event taking place in the second six months of that year, and where the interval between the events is at least four months and not more than eight months.

[14] *Technology* is used to describe the type of forensic DNA analysis performed in the laboratory, such as RFLP, STR, Y-STR, XSTR, SNP, microhaplotypes or mitochondrial DNA.

[15] *Method* is a combination of procedural steps used to perform a specific technical process. The method includes the validated steps, reagents, and critical instruments needed to perform the process or portion of a process. The same method may be conducted using different equipment (automated vs manual) when appropriately validated.

[16] *Methodology* refers to the categories of methods used to perform a stage of a DNA typing technology or technologies. For example, methodologies for STR technologies can include extraction, quantification, amplification, and detection.

13.1.4.1 Laboratories that employ technicians and/or use a team approach for casework examination may do so on external proficiency tests. However, each analyst shall be assigned a proficiency test to complete the interpretation and report the results.

13.1.4.1.1 If a methodology is performed by a technician or another analyst (other than the analyst assigned the proficiency test) on one test in the calendar year, the assigned analyst must perform the methodology on the other (second) test in the calendar year them self.

13.1.4.1.2 Technicians will ask a qualified analyst to interpret their serology test results before proceeding with DNA analysis, if applicable.

13.1.4.1.3 Technicians will mark the area in the test provider's reporting system (portal) indicating that no interpretations were made and include a note in any available comments section that they are completed the test as a technician and therefore no interpretations were made.

13.1.5 Individuals whose sole responsibility is technical review shall be proficiency tested in the technical review of each technology and typing test kit at least once per calendar year.[17]

13.1.5.1 The proficiency testing shall cover the CODIS core loci or CODIS core sequence ranges attempted for each technology at least once per calendar year.

13.1.5.2 If applicable, technical reviewers qualified to review modified Rapid DNA analysis shall be externally proficiency tested on the technical review of data generated by a Rapid DNA instrument model for each PCR STR typing test kit at least once per calendar year.

13.1.5.3 If the technical reviewer is a contract employee conducting technical reviews for an NDIS participating laboratory the proficiency testing shall be administered by an NDIS participating laboratory and shall be reviewed and approved by the technical manager of the NDIS participating laboratory for which the technical manager is conducting reviews.

13.1.6 Newly qualified individuals (analysts, technicians, and other personnel designated by the technical manager) shall undergo semi-annual external proficiency testing within eight months of their qualification date.

13.2 The OSBI forensic biology units shall use an external proficiency test provider that is accredited to the current applicable standard of the International Organization for Standardization and the applicable test is included on the proficiency test provider's scope of accreditation. The laboratory shall follow OSBI CSD Quality Manual 6.6.2 (Determining Suitability of External Providers) for external proficiency test providers. External proficiency testing shall be an open proficiency testing program and shall be submitted to the proficiency testing provider in order to be included in the provider's published external summary report.

13.2.1 If an analyst participates in a proficiency test provider's pre-distribution program, the pre-distribution test can be counted as one of the two external proficiency tests for the calendar year; however, the analyst must resubmit the pre-distribution test results during the general distribution testing phase for that specific test in order to be included in the provider's published external summary report.

[17] A qualified analyst proficiency tested in the specific technology may serve as a technical reviewer without needing to take an additional proficiency test as a technical reviewer.

13.3 For purposes of tracking compliance with the proficiency testing requirements, the OSBI forensic biology units define and consistently use the due date as the date that the proficiency test is performed.

13.4 In accordance with OSBI CSD QP 30 (Proficiency Tests), the OSBI forensic biology units shall maintain the following records for proficiency tests:

13.4.1 The test set identifier;

13.4.2 Identity of the analyst, and other participants, if applicable;

13.4.3 Date of analysis and completion;

13.4.4 Copies of all data and notes supporting the conclusions;

13.4.4.1 Analysts interpreting results for technicians in proficiency tests will document their conclusions in the technician's documentation.

13.4.4.2 Technicians will place their completed examination documentation into their individual folders on the OSBI QA server. They are not required to use the BEAST LIMS for completing their proficiency test since they are not qualified for casework and do not generate OSBI Criminalistics Examination Reports.

13.4.5 The proficiency test results;

13.4.6 Any discrepancies noted; and

13.4.7 Corrective actions taken.

13.5 The OSBI forensic biology units shall evaluate proficiency test results and shall include, at a minimum, the following criteria:

13.5.1 All reported genotypes, phenotypes, and/or sequences are correct or incorrect according to consensus results or are compliant with the laboratory's interpretation guidelines.

13.5.2 Inclusions and exclusions are correct or incorrect.

13.5.3 All reported uninterpretable results and/or inconclusive conclusions are compliant with written laboratory guidelines.

13.5.3.1 The technical leader shall review any inconclusive conclusion for compliance with laboratory guidelines.

13.5.4 All final proficiency tests shall be evaluated as satisfactory or unsatisfactory. A satisfactory grade is attained when there are no analytical errors for the DNA typing data or reported conclusions.

13.5.4.1 All discrepancies or errors, to include the occurrence of administrative errors, and subsequent corrective actions, as applicable, shall be documented. This includes retaining correspondence with an accrediting body's proficiency review committee, if applicable. Non-administrative discrepancies and errors will be handled in accordance with CWQM_14.

13.6 The following shall be informed of the results of the proficiency test and documented as per OSBI CSD QP 30 (Proficiency Tests):

13.6.1 The proficiency test participant(s)

13.6.2 The technical manager

13.6.3 The casework CODIS administrator in the event of non-administrative discrepancies that affect the typing results and/or conclusions.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

CWQM_14 CORRECTIVE ACTION (↑ Table of Contents)

14.1 The OSBI forensic biology units shall follow OSBI CSD QP 13 (Nonconforming Work), QP 14.1 (Class II Nonconforming Work), QP 14.2 (Corrective Action – Class III Nonconforming Work), and QP 14.3 (Corrective Action – Class IV Nonconforming Work), as well as OSBI Agency Policy #110 (OSBI Progressive Discipline), as needed, to address nonconformities detected in casework analysis, proficiency tests, testimony, and audits. Nonconformities not requiring a corrective action plan may be remediated with documented correction or other documentation. The aforementioned policies define when a nonconformity requires documentation and/or a corrective action plan.

14.1.1 Corrective action plans developed to evaluate and remediate a nonconformity shall be documented and include the elements listed below in 14.2. The technical manager shall have access to all final, completed corrective actions as applicable to casework, databasing and/or proficiency tests for every individual within the OSBI Forensic Biology Discipline.

14.2 The laboratory's documented corrective action plan shall include the identification (when possible) of the cause(s) of the nonconformity, corrective actions taken with timeframes (where applicable), and preventive measures taken (where applicable) to minimize its reoccurrence.

14.2.1 Corrective action plans shall be approved by the technical manager prior to implementation. If necessary, the technical manager has the authority to initiate, suspend and resume technical operations for the laboratory or an individual in accordance with CWQM_5.2.5.2.

14.2.2 The casework CODIS administrator shall be notified when the nonconformity impacts DNA records entered into CODIS. If necessary, the casework CODIS administrator, in consultation with the OSBI CSD director and quality manager, may terminate an analyst's or laboratory's participation in CODIS until the reliability and security of the computer data can be assured in the event an issue with the data is identified in accordance with CWQM_5.3.5.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI Agency Policies and Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_15 AUDITS (↑ Table of Contents)

15.1 The OSBI forensic biology units shall be audited annually, at a minimum, in accordance with the QAS. In addition, all internal audits shall be conducted in accordance with OSBI CSD QP 17 (Audits). The annual audits shall occur every calendar year and shall be at least six months and no more than eighteen months apart. The audit must entail the review of documentation since at least the last annual audit to assess compliance to the QAS.

15.2 At least once every two years, an external audit shall be conducted by one or more auditor(s) from a second agency(ies) who has/have successfully completed the FBI's DNA auditor training course. At least one auditor shall be or have been an analyst previously qualified in the laboratory's current DNA technologies and platforms.

15.2.1 Each analyst, technical reviewer, casework CODIS administrator, and technical manager shall have his/her education, experience, and training qualifications evaluated and approved during two successive, separate external audits. Approval of an individual's education, experience, and training qualifications shall be documented in the Audit Document.

15.2.1.1 An analyst or technical reviewer that receives additional qualification in an additional technology(ies), typing test kit(s), or platform(s) shall have the additional training qualifications evaluated and approved during one external audit. Approval of additional training qualifications shall be documented in the Audit Document.

15.2.2 Each validation study shall be evaluated and approved during one external audit. Approved validation studies shall be documented in the Audit Document.

15.3 Internal audits shall be conducted by an audit team that includes at least one auditor who has successfully completed the FBI's DNA auditor training course. At least one audit team member shall be or have been an analyst previously qualified in the laboratory's current DNA technologies and platforms.

15.4 Internal and external audits shall be conducted utilizing the QAS Audit Document in effect at the time of the audit.

15.5 Internal and external audit documentation and, if applicable, corrective action(s) shall be reviewed by the technical manager to ensure that findings, if any, were appropriately addressed and this review shall be documented.

15.5.1 Internal and external audit documentation, and if applicable, corrective action(s) shall be provided to the casework CODIS administrator.

15.5.2 For NDIS participating laboratories, all external audit documentation and laboratory responses shall be provided to the FBI within thirty days of laboratory receipt of the Audit Document or report.

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

Attachment(s):
None

Quality Manual

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards Audit for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

FBI Quality Assurance Standards Audit for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_16 PROFESSIONAL DEVELOPMENT (↑ Table of Contents)

16.1 The OSBI forensic biology units shall follow OSBI CSD QP 16.1 (Control of Records), OSBI CSD QP 19 (Training), and the requirements listed in this policy to ensure technical qualifications are maintained and documented through participation in continuing education.[18]

16.1.1 The technical manager, casework CODIS administrator, analyst(s), and technical reviewers shall stay abreast of topics relevant to the field of forensic DNA analysis by attending seminars, courses, professional meetings, or other documented lectures or classes in relevant subject areas for a minimum of eight cumulative hours each calendar year.[19]

16.1.1.1 The continuing education hours shall be documented. Attendance at a regional, national, or international conference with content including topics relevant to the field of forensic DNA analysis shall be deemed to provide a minimum of eight hours of continuing education.

16.1.1.2 Documentation of attendance, such as certificates, attendance lists, and/or travel documentation, shall be maintained.

16.1.1.3 With the exception of a regional, national, or international conference, documentation of content, such as agenda/syllabus, record of presentation content, and/or the curriculum vitae of the presenter, shall be maintained.

16.1.1.4 Continuing education based on multimedia or internet delivery shall be subject to the approval of the technical manager and documented.

16.1.2 Analysts and technicians shall participate in annual ongoing reading of scientific literature covering topics relevant to the field of forensic DNA analysis; laboratory support personnel (e.g. administrative technicians) are exempt, as this falls outside the scope of their duties.[20] The technical manager or Lead Analyst will approve and distribute applicable scientific literature ("literature reviews") each quarter for individuals to review before the next quarter or by the due date set by the technical manager or Lead Analyst. Individuals will verify completion of literature reviews by signing an associated completion form provided by the technical manager or Lead Analyst. All literature review documentation, including signed completion forms, will be maintained by the technical manager or Lead Analyst.

16.1.2.1 The laboratory shall maintain or have physical or electronic access to a collection of current books, reviewed journals, or other literature applicable to DNA analysis.

16.2 The OSBI forensic biology units shall follow OSBI CSD QP 32 (Testimony) to document the annual review of the testimony of each analyst.

16.2.1 OSBI CSD QP 32 (Testimony) and its associated attachments define the elements and mechanisms for testimony review.

16.2.2 The testimony review shall be documented and provided to the testifying individual.

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[18] Assignments and activities required as part of the laboratory's training program and/or that are required for establishing an individual's competency do not count toward the continuing education hours.

[19] An individual who completes the laboratory's initial training program within the calendar year is not expected to complete the 8 hours of continuing education until the next calendar year.

[20] Reading of scientific literature and subsequent lab-sponsored discussions (e.g. journal club, article presentation, etc.) do not count toward the continuing education hours required by 16.1.1.

16.2.2.1 Any deficiency and subsequent corrective actions, as applicable, shall be documented.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

CWQM_17 OUTSOURCING OWNERSHIP[21] (↑ Table of Contents)

17.1 A vendor laboratory performing forensic DNA analysis shall comply with the current QAS and the accreditation requirements of federal law. Except as provided by 17.2.2, failure to comply with the procedures listed herein will preclude the entry, searching or uploading of outsourced DNA data into CODIS. In addition, per OSBI CSD Quality Manual 6.6.2, any vendor laboratory utilized by the OSBI forensic biology multi-laboratory system (discipline) must comply with the accreditation requirements in O.S. 74-150.37 and be accredited through an organization that adheres to the ISO/IEC 17025 guidelines; a copy of the accreditation certificate shall be provided to the OSBI CSD quality manager.

17.1.1 As a NDIS participating laboratory, when outsourcing to a vendor laboratory (or if the criteria of ownership applies), the OSBI forensic biology discipline, or any unit therein, shall require the vendor laboratory to provide documentation of compliance with the QAS and the accreditation requirements of federal law. The OSBI forensic biology discipline technical manager shall review and retain the documentation of the vendor laboratory's compliance with the QAS and the accreditation requirements of federal law.

17.2 Except as provided in 17.2.1 and 17.2.2, the OSBI forensic biology discipline technical manager shall document approval of the technical specifications of the outsourcing agreement with the vendor laboratory before it is awarded, and the date of the technical manager's documented approval and/or the documented prior approval as specified in 17.2.1 must be maintained. In addition, when bid specifications are written by any individual other than the technical manager, the technical manager shall be consulted for input and approval.[22] The applicable OSBI forensic biology unit(s) shall also notify customers in writing, prior to shipment of evidence, when utilizing a vendor laboratory for serology and/or DNA analyses.

17.2.1 A vendor laboratory that is performing forensic DNA analysis on behalf of a law enforcement agency (other than the OSBI) or other entity for the purposes of ownership by the OSBI laboratory, shall not initiate analysis until written approval has been obtained from the OSBI forensic biology discipline technical manager.

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[21] Ownership applies if any of the following will occur, including in situations where a vendor laboratory subcontracts:

1. The OSBI will use any samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing (e.g. a vendor laboratory prepares an extract that will be analyzed by the OSBI);
2. The OSBI will interpret the data generated by the vendor laboratory;
3. The OSBI will issue a report describing or drawing conclusions on the results of the DNA analysis performed by the vendor laboratory; or
4. The OSBI will enter or search a DNA profile in CODIS from data generated by the vendor laboratory.

[22] Bid specifications should include the following requirements, at a minimum:

- Prior to conducting DNA analysis, the vendor laboratory must guarantee compliance with confidentiality requirements set for by the OSBI.
- The vendor laboratory shall only use procedures approved by the OSBI forensic biology discipline technical manager for forensic DNA analysis.
- DNA analysis will be attempted at all 20 CODIS core loci.
- All data generated during analysis by a vendor laboratory is property of the State of Oklahoma and will be returned to the OSBI after analysis is complete.
- The vendor laboratory is responsible for providing evidence analysis and testimony regarding their analyses.
- OSBI personnel may conduct an audit at the discretion of the OSBI forensic biology discipline technical manager, if necessary.

17.2.2 For rare instances where the OSBI forensic biology discipline, or any unit therein, is requested to take ownership and no outsourcing agreement exists between either the law enforcement agency, the vendor laboratory or the OSBI, the OSBI forensic biology discipline technical manager shall document the following prior to acceptance of ownership of product(s) of forensic DNA analyses from the vendor laboratory:

17.2.2.1 Approval of the casework CODIS administrator and written permission from the NDIS Custodian for any scenario that involve CODIS entry or searching;

17.2.2.2 Approval of the technical specifications of testing; and

17.2.2.3 Review the documentation of or conduct an on-site visit of the vendor laboratory. The on-site visit shall have been within eighteen months of the conducted analysis and in accordance with 17.4.2.

17.3 The OSBI forensic biology discipline, or any unit therein, will verify the integrity of the DNA data received for the purposes of taking ownership of DNA data from a vendor laboratory by performing a technical review (i.e. ownership review) following the procedures described in CWQM_12 (Review) and completing a technical review form.

17.3.1 Prior to the search of DNA data in SDIS, an analyst, casework CODIS administrator or technical reviewer in the OSBI forensic biology discipline (or OSBI contract employee) shall review the DNA data to verify specimen eligibility and the correct specimen category for entry into CODIS.

17.3.2 Prior to the upload of DNA data to SDIS or the reporting of search results, an analyst or technical reviewer in the OSBI forensic biology discipline (or OSBI contract employee) who is qualified in the technology, platform and typing test kit used to generate the data and participates in the OSBI laboratory's proficiency testing program shall perform a technical review (ownership review) of the DNA data from the vendor laboratory.

17.3.2.1 If the proficiency testing is administered by another NDIS participating laboratory, such as for a contracted technical reviewer, the OSBI forensic biology discipline technical manager (i.e. the NDIS participating laboratory for which the reviewer is conducting ownership reviews) shall review and approve the reviewer's participation in an NDIS participating laboratory's proficiency testing program.

17.3.3 Except as provided in 17.3.4, the technical review (ownership review) shall include the following elements:

17.3.3.1 A review of all DNA types that the OSBI forensic biology discipline, or any unit therein, will take ownership of to verify that they are supported by the raw and/or analyzed data (electropherograms or images).

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

17.3.3.3 A review of the final report (if provided) to verify that the results/conclusions are supported by the data. Each item (or its probative fraction) tested by the vendor laboratory must be addressed.

17.3.3.4 For samples to be entered into CODIS, verification of the DNA types, eligibility, and the correct specimen category.

17.3.3.4.1 Verification of eligibility must be performed by a current CODIS user.

17.3.4 If applicable, for an NDIS participating laboratory that outsources to a vendor laboratory performing Rapid DNA analysis on casework reference samples using an NDIS approved Rapid DNA System, the ownership review for data generated by the Rapid DNA System shall include:

17.3.4.1 A review of the final report (if provided) to verify that the results/conclusions are supported by the Rapid DNA System data.

17.3.4.2 For samples to be entered into CODIS, verification of the eligibility and the correct specimen category.

17.3.4.2.1 Verification of eligibility must be performed by a current CODIS user.

17.3.4.3 A review of the data associated with applicable Rapid DNA System performance checks.

17.4 When the OSBI forensic biology discipline, or any unit therein, outsources DNA sample(s) to a vendor laboratory or accepts ownership of DNA data from a vendor laboratory, an on-site visit(s) of the vendor laboratory shall be performed and documented, provided, however, that an on-site visit shall not be required when only technical review services are being provided.[23] Documentation must include the date the visit was performed, who performed the visit, and a summary of the visit in an OSBI memorandum copied to the OSBI CSD director. The procedure to perform an on-site visit shall include, at a minimum, the following elements:

17.4.1 A documented initial on-site visit, to assess the vendor laboratory's ability to perform analysis on outsourced casework, prior to the vendor laboratory's beginning of casework analysis for the OSBI forensic biology discipline, or any unit therein.

17.4.1.1 The on-site visit shall be performed by the OSBI forensic biology discipline technical manager, or a designated employee of a NDIS participating laboratory, who is a qualified or previously qualified analyst in the technology, platform and typing test kit used to generate the DNA data. Alternatively, the OSBI forensic biology discipline technical manager shall evaluate and approve an on-site visit coordinated by a designated FBI employee.

17.4.2 If the outsourcing agreement extends beyond one year, an annual on-site visit shall be required by the OSBI forensic biology discipline technical manager, or a designee, as described above. Each annual on-site visit shall occur every calendar year and shall be at least six months and no more than eighteen months apart.

17.4.2.1 The OSBI forensic biology discipline, or any unit therein, may accept an on-site visit conducted by another NDIS participating laboratory using the same technology, platform or typing test kit for the generation of the DNA data, or coordinated by a designated FBI employee, and the OSBI forensic biology discipline technical manager shall review and approve such on-site visit.

Attachment(s):
None

[23] While an on-site visit is not required when only technical review services are being provided, the OSBI forensic biology discipline technical manager shall evaluate how and where such services are being performed and document their approval to ensure compliance with the QAS, Standard 11.3.

Quality Manual

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

SER_1 HEMASTIX® (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

1.1 **Scope**
Presumptive test for the presence of blood.

1.2 **Reagents & Supplies**

1.2.1 Hemastix® (Bayer Healthcare Model 2190 or another equivalent product)

1.2.2 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:

1.3.1.1 A positive control made up of known blood.

1.3.1.2 A negative control consisting of deionized water 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 technical manager, Lead Analyst, and unit supervisor, and the lot should be removed from casework use.

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

1.3.3.1 Hemastix®

1.3.3.2 Deionized water

1.3.3.3 Known blood used for positive control

1.4 **Individual Steps of the Procedure**

1.4.1 **Dry Method**

1.4.1.1 Lightly brush the dry reagent pad of the Hemastix® on the suspected stain.

1.4.1.2 Add deionized water to the reagent pad.

1.4.1.3 Observe reagent pad within 5 seconds for the presence of a color change.

1.4.2 **Wet Method**

1.4.2.1 Add deionized water to the reagent pad of the Hemastix®.

1.4.2.2 Lightly brush the moistened reagent pad of the Hemastix® on the suspected stain.

1.4.2.3 Observe reagent pad and/or stain within 5 seconds for the presence of a color change.

1.5 **Interpretation of Results**

1.5.1 Positive result: A blue or green color develops on the reagent pad or on the stain.

1.5.2 Negative result: No blue or green color develops within 5 seconds.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_Hemastix**

Reference(s):
OSBI Forensic Biology Units' Training Manual

SER_2 TAKAYAMA (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

2.1 **Scope**
Confirmatory test for the presence of blood.

2.2 **Reagents & Supplies**

2.2.1 10% sodium hydroxide

2.2.2 Saturated glucose solution

2.2.3 Pyridine

2.2.4 0.05M dithiothreitol (DTT)

2.2.5 Deionized water

2.2.6 Slide and cover slip

2.3 **Equipment**

2.3.1 Microscope

2.3.2 Hot plate (Option A only)

2.4 **Reagent Preparation**
Prepare the Takayama reagent according to either Option A or Option B combination:

2.4.1 **Takayama Reagent Option A**

2.4.1.1 1 part 10% sodium hydroxide

2.4.1.2 1 part saturated glucose solution

2.4.1.3 1 part pyridine

2.4.1.4 2 parts deionized water

2.4.2 **Takayama Reagent Option B**

2.4.2.1 1 part 10% sodium hydroxide

2.4.2.2 1 part saturated glucose solution

2.4.2.3 1 part pyridine

2.4.2.4 2 parts 0.05M dithiothreitol

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 to verify the function of the reagents and the microscope:

2.5.1.1 A positive control made up of known blood and Takayama reagent.

2.5.1.2 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 technical manager, Lead Analyst, and unit supervisor, and the lot should be removed from casework use.

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**

2.6.1 Place a small sample from the suspected bloodstain on a microscope slide and place a small cover slip on the sample.

2.6.2 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.

2.6.3 If using Option A, heat the slide gently until there is a change in the color of the material under the cover slip.

2.6.4 Observe the slide under the microscope.

2.7 **Interpretation of Results**

2.7.1 Positive result: Formation of pink to red, blade, needle or rhomboidal shaped crystals.

2.7.2 Negative result: No formation of characteristic crystals.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_Takayama**

Reference(s):
OSBI Forensic Biology Units' Training Manual

SER_3 RSID™-BLOOD (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

3.1 **Scope**
Human blood detection test.

3.2 **Reagents & Supplies**

3.2.1 RSID™-Blood cassettes

3.2.2 RSID™-Blood Running Buffer

3.2.3 RSID™-Blood Extraction Buffer

3.3 **Equipment**

3.3.1 Mechanical pipettor

3.3.2 Vortex

3.3.3 Centrifuge

3.4 **Controls**

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

3.4.1.1 A positive control, prepared as follows:

3.4.1.1.1 Apply ~50µL of human blood to a sterile cotton swab.

3.4.1.1.2 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.

3.4.1.1.3 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 1 minute at ~13200 RPM, vortex thoroughly.

3.4.1.1.4 Take ~20µL of the blood extract and place in a fresh microcentrifuge tube and add ~80µL of RSID™-Blood Running Buffer to bring to a total volume of ~100µL, vortex thoroughly.

3.4.1.1.5 Load the ~100µL of the sample into the cassette window.

3.4.1.1.6 Record results at ~10 minutes.

3.4.1.2 A negative control, prepared as follows:

3.4.1.2.1 Apply ~50µL of deionized water to a sterile cotton swab.

3.4.1.2.2 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.

3.4.1.2.3 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 1 minute at ~13200 RPM, vortex thoroughly.

3.4.1.2.4 Take ~20µL of the extract and place in a fresh microcentrifuge tube and add ~80µL of RSID™-Blood Running Buffer to bring to a total volume of ~100µL, vortex thoroughly.

3.4.1.2.5 Load the ~100µL of the sample into the cassette window.

3.4.1.2.6 Record results at ~10 minutes.

3.4.2 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 technical manager, Lead Analyst, and unit supervisor, and the lot should be removed from casework use.

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

3.4.3.1 RSID™-Blood cassette

3.4.3.2 RSID™-Blood Extraction Buffer

3.4.3.3 RSID™-Blood Running Buffer

3.4.3.4 Known human blood used for positive control

3.5 **Individual Steps of the Procedure**

3.5.1 Take a cutting from the swab or material and place it in a microcentrifuge tube.

3.5.2 Add ~50µL of RSID™-Blood Extraction Buffer, vortex thoroughly and incubate at room temperature for ~1 hour.

3.5.3 Following the extraction, remove the cutting from the tube and place into a tube basket inside the tube or the perforated tube cap and centrifuge the sample for 1 minute at ~13200 RPM, vortex thoroughly.

3.5.4 Take ~20µL of the extract and place in a fresh microcentrifuge tube and add ~80µL of RSID™-Blood Running Buffer to bring to a total volume of ~100µL, vortex thoroughly.

3.5.5 Add the ~100µL of extract to the sample cassette window.

3.5.6 Record the results at ~10 minutes.

3.6 **Interpretation of Results**

3.6.1 Positive result: Formation of lines at both control and test lines at ~10 minutes.

3.6.2 Negative result: Formation of a line at the control region only at ~10 minutes.

3.7 **Notes**

3.7.1 This test is to be used at analyst's discretion. In addition, this test is to be used only when the case circumstances suggest it necessary (i.e. question of human versus non-human blood).

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_RSID-Blood**

Reference(s):
OSBI Forensic Biology Units' Training Manual

SER_4 AP SPOT TEST (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

4.1 **Scope**
Presumptive test for the presence of semen.

4.2 **Reagents & Supplies**

4.2.1 AP Spot Test solution A

4.2.2 AP Spot Test solution B

4.2.3 Deionized water

4.2.4 Filter paper and/or sterile swabs

4.3 **Reagent Preparation**
Prepare the AP Spot Test solution by thawing and combining solutions A and B together.

4.4 **Controls**

4.4.1 The following controls are required to be run and recorded once per lot on each day that the AP Spot Test is used:

4.4.1.1 A positive control made up of known semen with AP Spot Test solution added.

4.4.1.2 A negative control consisting of AP Spot Test solution only.

4.4.2 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 technical manager, Lead Analyst, and unit supervisor, and the lot should be removed from casework use.

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

4.4.3.1 AP Spot Test solution A

4.4.3.2 AP Spot Test solution B

4.4.3.3 Deionized water

4.4.3.4 Known semen used for positive control

4.5 **Individual Steps of the Procedure**

4.5.1 **Rubbing Method**

4.5.1.1 Wet filter paper or swab with deionized water and rub suspected stain.

4.5.1.2 Add ~3 drops of AP Spot Test solution to the filter paper or swab.

4.5.1.3 Observe paper or swab for the presence of a color within 2 minutes.

4.5.2 **Cutting Method**

4.5.2.1 Take a cutting of suspected stain and place in well on plate or on a piece of filter paper.

4.5.2.2 Add ~3 drops of AP Spot Test solution to well or filter paper.

4.5.2.3 Observe well or paper for the presence of a color within 2 minutes.

4.6 **Interpretation of Results**

4.6.1 Positive result: A purple color develops within 2 minutes.

4.6.2 Negative result: No color change is observed or any other color develops besides purple within 2 minutes.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_APSpot_Test**

Reference(s):
OSBI Forensic Biology Units' Training Manual

SER_5 SERATEC® (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

5.1 **Scope**
Confirmatory test for the presence of p30, a component of seminal fluid, using immunoassay.

5.2 **Reagents & Supplies**

5.2.1 Phosphate Buffered Saline (PBS)

5.2.2 P30 test device (Seratec® PSA SemiQuant or other approved device)

5.3 **Equipment**

5.3.1 Mechanical pipettor

5.3.2 Centrifuge

5.4 **Sample Preparation**

5.4.1 Refer to CASE_3 (Serology Analysis) for sample handling guidance, as applicable for this method, including when and how to combine samples for testing.

5.5 **Controls**

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

5.5.1.1 A positive control of ~1:100 dilution of reconstituted semen standard or known semen in PBS.

5.5.1.2 A negative control consisting of PBS.

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

5.5.3 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 technical manager, Lead Analyst, and unit supervisor, and the lot should be removed from casework use.

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

5.5.4.1 P30 test device (Seratec® PSA SemiQuant or other approved device)

5.5.4.2 Phosphate Buffered Saline (PBS)

5.5.4.3 Semen standard / known semen used for positive control

5.6 **Individual Steps of the Procedure**

5.6.1 Place a cutting of a suspect semen stain or swab cutting(s) into a microcentrifuge tube. If sample is liquid, reference step 5.6.5 below.

5.6.2 Add 250µL to 750µL of phosphate buffered saline to the tube.

5.6.3 Allow the sample to soak for ~30 minutes. If sperm search is also to be performed, cloth samples should be sonicated for ~15 minutes. Analysts may use discretion for sonicating other sample types.

5.6.4 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 ~5 minutes at maximum speed.[24]

5.6.5 Add ~200µL of supernatant to the sample well of the test device.[25] If the sample is a liquid, 200µL or a dilution of the sample may be used.

5.6.6 Observe testing device at ~10 minutes.

5.7 **Interpretation of Results**

5.7.1 Positive result: Formation of three lines in the result well, to include a test result line (T), internal standard line, and control line (C), at ~10 minutes.

5.7.2 Negative result: Formation of two lines in the result well, to include an internal standard line and control line (C), at ~10 minutes.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_Seratec**

Reference(s):
OSBI Forensic Biology Units' Training Manual

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

[25] May use supernatant transferred to a tube for p30 testing from the SER_6 procedure.

SER_6 MICROSCOPIC IDENTIFICATION OF SPERMATOZOA (↑ Table of Contents)

Quick links:
CASE_3 (Serology Analysis)
Procedure A
Procedure B

6.1 **Scope**
Confirmatory test for the presence of seminal fluid. Procedure A or B may be used at analyst discretion depending on sample type.

6.2 **Reagents & Supplies**

6.2.1 Phosphate Buffered Saline (PBS)

6.2.2 Christmas Tree Stain: Nuclear Fast Red stain (Stain A) and Picroindigocarmine stain (Stain B)

6.2.3 Methanol or ethanol

6.2.4 Deionized water

6.2.5 Permount

6.2.6 Microscope slide and cover slip

6.3 **Equipment**

6.3.1 Microscope

6.3.2 Hot plate

6.3.3 Centrifuge

6.4 **Sample Preparation**

6.4.1 Refer to CASE_3 (Serology Analysis) for sample handling guidance, as applicable for this method, including when and how to combine samples for testing.

6.5 **Controls**

6.5.1 Known human sperm cells previously stained with Christmas Tree Stain should be viewed and recorded prior to casework to verify the functionality of the reagents and microscope.

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

6.5.2.1 Phosphate Buffered Saline (PBS)

6.5.2.2 Christmas Tree Stain (Stain A/red and Stain B/green)

6.5.2.3 Permount

6.5.2.4 Known human sperm slide used for positive control

6.6 **Individual Steps of the Procedure**

6.6.1 Place a cutting of a suspect semen stain, swab cutting(s), or liquid into a microcentrifuge tube.

6.6.2 Add 250µL to 750µL of phosphate buffered saline to the tube and extract for ~30 minutes.

6.6.3 During the extraction, cloth samples should be sonicated for ~15 minutes. Analysts may use discretion for sonicating other sample types.

6.6.4 Following the extraction, remove the cutting(s) 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.

6.6.5 Perform one of the following options:

6.6.5.1 Option A: Remove the supernatant and place into another tube to use for p30 testing (see SER_5); reconstitute the pellet in approximately 10µL of phosphate buffered saline, or

6.6.5.2 Option B: Remove all but ~10µL of supernatant and place into another tube to use for p30 testing (see SER_5); mix the remaining ~10µL of supernatant with the pellet.

6.6.6 Remove all remaining sample and apply to a microscope slide.

6.6.7 Heat fix the smear to the slide by heating with a hot plate.

6.6.8 Stain the smear with one or more drops of Nuclear Fast Red stain (Stain A) for 5 to 15 minutes.

6.6.9 Rinse the slide with deionized water to remove any excess stain.

6.6.10 Stain the smear with one or more drops of Picroindigocarmine stain (Stain B) for 5 to 30 seconds.

6.6.11 Rinse the slide with either ethanol or methanol to remove any excess stain.

6.6.12 Allow the slide to air dry or place the slide on a hot plate and allow the slide to completely dry.

6.6.13 Using Permount in a hood, attach a cover slip to the slide.

6.6.14 View the slide microscopically (confirm spermatozoa with a 40x objective) and record the absence or presence of spermatozoa, Vernier numbers, and slide number (and well designation, if applicable).

6.7 **Interpretation of Results**

6.7.1 Positive result: Intact or partial sperm cell(s) observed.

6.7.2 Negative result: No sperm cells observed.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_SER_Xmas_Tree_Stain**

Reference(s):
OSBI Forensic Biology Units' Training Manual

SER_7 HAIR ANALYSIS (↑ Table of Contents)

Quick link:
CASE_3 (Serology Analysis)

7.1 Scope

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

7.2 Reagents & Supplies

7.2.1 Deionized water

7.2.2 Microscope slide & coverslip

7.3 Equipment

7.3.1 Microscope

7.4 Individual Steps of the Procedure

7.4.1 **Macroscopic Examination**

Visually examine the hair(s). The following macroscopic characteristics should be documented before performing a microscopic analysis:

7.4.1.1 Approximate color and shade.

7.4.1.2 Hair form, such as straight, curved, wavy, loose curl, tight curl, etc.

7.4.1.3 Approximate length to the nearest centimeter.

Note, when analyzing a large number of hairs, macroscopic characteristics can be used to help with sample selection, if desired. The analyst may group hairs based on similar macroscopic characteristics, and then microscopic analysis can be performed on a select number of hairs from the group(s). In the event that microscopic analysis shows the hairs chosen in the sample selection 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 all of the hairs in the group based on the microscopic analysis of only a few hairs, and the report wording must reflect this.

7.4.2 **Microscopic Examination**

If a microscopic analysis is necessary, wet-mount the hair(s) using deionized water and view with the aid of a microscope. The following characteristics shall be identified (as appropriate) and documented in the case notes by written description and/or photograph:

7.4.2.1 Characteristics of the medulla such as diameter with relation to the whole hair.

7.4.2.2 Any other distinguishing characteristics (e.g. apparent artificial color).

7.4.2.3 The presence or absence of a root.

7.4.2.4 If the root is present, whether there is cellular material present.

7.4.3 **Determination of Origin (Human v. Non-Human)**
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.

7.4.3.1 Color/pigmentation:

7.4.3.1.1 For human hairs, color will be relatively consistent along the shaft aside from chemical treatments.

7.4.3.1.2 For human hairs, pigment is typically distributed evenly or distributed toward the cuticle, except in red hair where the distribution is toward the medulla.

7.4.3.1.3 Animal hairs can exhibit color banding that varies along the shaft.

7.4.3.2 Medulla:

7.4.3.2.1 Human hair medulla is typically <⅓ of the diameter of the hair shaft and amorphous in structure.

7.4.3.2.2 Animal hair medulla is typically >⅓ of the diameter of the hair shaft.

7.4.3.2.3 Human hair medulla can be fragmentary (trace), interrupted (discontinuous), continuous or absent. This is not always consistent between hairs from one area (e.g. head) and can vary for each person.

7.4.3.2.4 Animal hair medulla can be lattice, uniserial, multiserial (corncob), cellular or vacuolated.

7.4.3.3 Root shape:

7.4.3.3.1 Human hair root shape is bulbous.

7.4.3.3.2 Animal hair root shape is characteristic for some animals (e.g. dog has spade shape, deer has wine glass shape, etc.).

7.4.3.4 Diameter:

7.4.3.4.1 Human hair diameter variation is relatively moderate with a shaft diameter of ~30 to 110µm and an average of ~80µm.

7.4.3.4.2 Animal hair diameter might have some variation along the shaft (distinct in some animals) with a shaft diameter ranging from ~5µm to 2mm.

7.4.3.5 Scales:

7.4.3.5.1 Human hair has only imbricate scales.

7.4.3.5.2 Animal hairs have varied scale structures.

7.5 **Interpretation of Results**

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

7.5.2 If the analyst cannot determine whether a hair is 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 the hair for DNA if nuclear material is present).

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

7.5.4 If a hair is to be analyzed for nuclear DNA, then it must be handled in accordance with consumption policies and photographed microscopically with no less than a 10x objective prior to any destructive analysis being performed.

Attachment(s):
None

Reference(s):
OSBI Forensic Biology Units’ Training Manual

DNA_1 Y-SCREEN ASSAY (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)

1.1 **Scope (Extraction Method)**
Confirmatory DNA test for the presence of human male DNA (“Direct to DNA” approach).

1.2 **Reagents & Supplies**

1.2.1 PrepFiler™ Lysep Column/hingeless sample tube assembly

1.2.2 1N Sodium Hydroxide

1.2.3 Glacial Acetic Acid

1.2.4 TE^{-4}

1.2.5 Quantifiler™ Trio DNA Quantification Kit

1.2.6 Quantifiler™ Automation Enhancer Kit

1.2.7 96-well optical reaction plate

1.2.8 Optical adhesive cover

1.2.9 Canned air (optional)

1.3 **Equipment**

1.3.1 Mechanical pipettors

1.3.2 Centrifuge

1.3.3 Vortex

1.3.4 Eppendorf ThermoMixer® C

1.3.5 Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System

1.4 **Reagent Preparation**

1.4.1 Prepare the Quantifiler™ Working Lot by adding the Quantifiler™ Automation Enhancer Kit to the THP PCR Reaction Mix according to the Quantifiler™ Reagents OSBI Chemical Receipt/Function Verification Worksheet (**QCR_DNA_QF_Reagents**), if necessary.

1.4.2 Prepare the quantitation standards according to the Quantifiler™ Trio Quantitation Standards OSBI Chemical Formulation/Function Verification Worksheet (**QCR_DNA_Quant_Standards**), if necessary.

1.5 **Sample Preparation**

1.5.1 The Y-screen assay should only be used to screen intimate orifice swabs included in sexual assault kits or collected by the medical examiner that do not require consumption.

1.5.2 Refer to CASE_4 (DNA Analysis) for guidance on appropriate sample handling for this method, including when and how to combine samples for testing.

1.6 Controls

1.6.1 All extraction sets require the concurrent processing of at least one reagent blank.

1.6.2 Each quantitation plate requires a Quantitation Negative Control (QNC)/Non-Template Control (NTC).

1.6.3 Associated reagent blank(s) will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

1.6.4.1 1N Sodium Hydroxide

1.6.4.2 Glacial Acetic Acid

1.6.4.3 TE^{-4}

1.6.4.4 Quantifiler™ Working Lot

1.6.4.5 Quantifiler™ Trio DNA Quantification Kit

1.6.4.6 Quantifiler™ Automation Enhancer Kit

1.6.4.7 Quantifiler™ Trio Quantitation Standards

1.7 Individual Steps of the Procedure

1.7.1 Place ~1/10 of swab cutting or cuttings, as appropriate, into a labeled PrepFiler™ Lysep Column/hingeless sample tube assembly.

1.7.2 Add 100µL of 1N NaOH to each sample; perform this step in a hood. Ensure all cuttings are immersed in liquid.

1.7.3 Incubate on a ThermoMixer® at 80°C and 750 RPM for 10 minutes.

1.7.4 Remove samples from ThermoMixer® and allow them to cool (to the touch). (Opening the cap when the tube is hot releases some pressure, which could cause splashing.)

1.7.5 Centrifuge samples for 2 minutes at 12,000 RPM to transfer the lysate to the sample tubes.

1.7.5.1 If the membrane clogged, perform an additional spin at maximum speed for a longer length of time until the lysate is in the bottom of the tube. Note, a small amount of lysate will remain in the Lysep Column due to the configuration of the column; an additional centrifugation step is not needed in this instance if there is volume in the bottom of the tube.

1.7.6 Discard the Lysep Column containing the cutting.

1.7.7 Add 4µL of glacial acetic acid to each tube to neutralize the lysis; perform this step in a hood.

1.7.8 Dilute by adding 400µL of TE^{-4} directly to each neutralized sample (1:5 dilution) and transfer the total sample volumes to new 1.5mL tubes with caps, or, alternatively, transfer each neutralized sample to new 1.5mL tubes with caps that contain 400µL of TE^{-4} to dilute each neutralized sample (1:5 dilution).

1.7.9 Vortex and spin down.

1.7.10 Proceed directly to quantification and quantify the samples according to the procedure in DNA_4.6.

1.8 **Interpretation of Results**

1.8.1 The QNC should be "undet." during quantitation (no signal). If the QNC displays a signal, it will be marked as "Unknown" and reanalyzed to determine the amount of DNA detected. The technical manager and/or Lead Analyst, shall be notified and determine if the plate is acceptable or if it will be re-setup and rerun.

1.8.2 Verify that all quantification standards performed properly.

1.8.3 Check samples and controls for possible inhibition or improper amplification including the IPC C_T for each sample. If observed, the sample may need to be re-quantitated or re-analyzed.

IPC C_T flag triggered?	Degradation Index	Quality Index Interpretation†
No	<1	Typically indicates that DNA is not degraded or inhibited.
	1 to 10	Typically indicates that DNA is slightly to moderately degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.
	>10 or blank (no value)	Typically indicates that DNA is significantly degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.
Yes	<1	Although theoretically possible, this result is unlikely because PCR inhibitors in sufficient concentration to trigger the IPC C_T flag typically would affect the large autosomal target as well.
	>1 or blank (no value)	Typically indicates that the DNA is affected by degradation and/or PCR inhibition.

† These are general guidelines that may not apply to all samples depending on the inhibitors present, the varying quantity of contributor DNA in mixed samples, and the STR kit used.

1.8.3.1 When encountering an elevated IPC C_T value associated with a Y-screen assay sample, determine if it appears to be the result of elevated (female) DNA present or due to a possible unknown PCR inhibitor affecting the sample by evaluating the IPC amplification plot and comparing the IPC C_T value of the sample to the IPC C_T values of the quantification standards with similar concentrations. Inhibition is generally assessed as having an IPC C_T value of undetermined or elevated by >1 C_T relative to the IPC C_T values of the quantification standards on the same plate with similar concentrations.

1.8.4 Reagent blanks will be handled in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

1.8.5 Generally, human DNA should be detected in the small autosomal target for both Y-screen positive and negative assay results. If no human DNA is detected in the small autosomal target, the sample may need to be re-quantitated or re-analyzed.

1.8.6 Positive result: Human male DNA detected for the Y target (> 0ng/µL).

1.8.7 Negative result: No human male DNA detected for the Y target, and no reaction failure due to inhibition (acceptable IPC C_T).

1.8.8 Inconclusive result: Reaction failure due to inhibition (IPC C_T for a sample or amplification curve indicate that the PCR assay was inhibited).

1.9 **Notes**

1.9.1 Steps taken to any samples must also be performed on an associated reagent blank per CWQM_9.5.1 (Analytical Procedures).

1.9.2 Smaller aliquots from stock solutions of 1N NaOH and Glacial Acetic Acid shall be used when performing the Y-screen assay. All aliquots must be stored appropriately and in a hood at all times (not in individual work areas).

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_YS_NaOH**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_YS_C2-H4-O2**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_QF_Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Quant_Standards**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Quant_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Ext_Quant_Setup_Workbook_v1.0**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Eppendorf ThermoMixer® C Operating Manual

Life Technologies™ Quantifiler™ HP and Trio DNA Quantification Kits User Guide

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (for Human Identification) User Guide for use with: HID Real-Time PCR Analysis Software v1.3

Applied Biosystems™ HID Real-Time PCR Analysis Software Version 1.3 User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

SWGDAM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits

OSBI Forensic Biology Units' Training Manual

DNA_2 STANDARD EXTRACTION & ISOLATION OF DNA (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)
Standard Extraction of DNA Using PrepFiler *Express*™ Kit (Lysate Preparation)
Isolation of DNA Using the AutoMate *Express*™ Forensic DNA Extraction System
Standard Extraction of DNA from Bone & Teeth Using PrepFiler *Express*™ BTA Kit
Standard Extraction of DNA from Adhesive Sample Types Using PrepFiler *Express*™ BTA Kit

2.1 **Scope (Extraction Method)**
Extraction and isolation of DNA from all samples, except those requiring a differential extraction, using the PrepFiler *Express*™ and/or PrepFIler *Express* BTA™ Forensic DNA Extraction Kit(s) and the AutoMate *Express*™ Forensic DNA Extraction System.

2.2 **Reagents & Supplies**

2.2.1 1M dithiothreitol (DTT)

2.2.2 Proteinase K

2.2.3 PrepFiler *Express*™ and/or PrepFiler *Express* BTA™ Forensic DNA Extraction Kits

2.3 **Equipment**

2.3.1 Mechanical pipettors

2.3.2 Centrifuge

2.3.3 Vortex

2.3.4 Eppendorf ThermoMixer® C

2.3.5 AutoMate *Express*™ Forensic DNA Extraction System

2.4 **Sample Preparation**
Refer to CASE_4 (DNA Analysis) for guidance on sample types and appropriate extraction kit to use.

2.5 **Controls**

2.5.1 All extraction sets require the concurrent processing of at least one reagent blank.

2.5.2 Associated reagent blanks will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

2.5.3 Requirement for lot numbers/expirations listed in case record (as applicable):

2.5.3.1 1M dithiothreitol (DTT)

2.5.3.2 Proteinase K

2.5.3.3 PrepFiler *Express*™ and/or PrepFiler *Express* BTA™ Forensic DNA Extraction Kits

2.6 Individual Steps of the Procedure

2.6.1 Standard Extraction of DNA Using PrepFiler *Express*™ Kit (Lysate Preparation)

2.6.1.1 Preheat the ThermoMixer® to 70°C.

2.6.1.2 Place sample/substrate into a PrepFiler™ Lysep Column/hingeless sample tube assembly.

2.6.1.3 Add the following reagents to each sample:

2.6.1.3.1 500µL of PrepFiler™ Lysis Buffer

2.6.1.3.2 5µL of 1M DTT

2.6.1.4 Incubate on a ThermoMixer® at 70°C and 750 RPM for 40 minutes.

2.6.1.5 Centrifuge the column/tube assembly for 2 minutes at ~10400 RPM to transfer the lysate into the sample tube.

2.6.1.6 Remove the Lysep Column containing the substrate from the sample tube and retain, if necessary, or discard.

2.6.1.7 If the volume of sample lysate collected in the sample tube is less than 300µL, centrifuge the column/tube assembly for an additional 5 minutes.

2.6.1.7.1 If the volume is still less than 300µL, add PrepFiler™ Lysis Buffer to bring the lysate volume to ~300µL.

2.6.1.8 If a pellet is present in the sample tube, transfer the clear lysate to a new PrepFiler™ sample tube.

2.6.1.9 If any salt precipitation is observed in the sample tube, 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 that contains precipitate on the AutoMate *Express*™ Forensic DNA Extraction System, or instrument crash, tip clogging, and/or filter wetting may occur.

2.6.1.10 Proceed directly to automated extraction run as described in step 2.6.2 below.

2.6.2 Isolation of DNA Using the AutoMate *Express*™ Forensic DNA Extraction System

Setup and run the AutoMate *Express*™ Forensic DNA Extraction System as follows:

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

2.6.2.2 Press start and open the door.

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

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

2.6.2.4.1 Row S (fourth row): Load with PrepFiler™ sample tubes containing lysate.

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

2.6.2.4.3 Row T1 (second row): Leave empty.

2.6.2.4.4 Row E (first row): Load with labeled PrepFiler™ elution tubes with caps open and secured.

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

2.6.2.6 Select 50uL elution volume, if applicable.

2.6.2.7 Press Start.

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

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

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

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

2.6.3 **Standard Extraction of DNA from <u>Bone & Teeth</u> Using PrepFiler *Express*™ BTA Kit (Lysate Preparation)**

2.6.3.1 Preheat the ThermoMixer® to 56°C.

2.6.3.2 Clean the bone or tooth to remove any adhered tissue.

2.6.3.3 Prepare a uniform bone or tooth powder using standard laboratory procedures (reference CASE_4 (DNA Analysis)) or in consultation with the technical manager, and/or Lead Analyst.

2.6.3.4 Transfer ~50mg of powdered bone or tooth into a new PrepFiler™ Bone and Tooth Lysate Tube.

2.6.3.5 Add the following reagents to each sample:

2.6.3.5.1 220µL of PrepFiler™ BTA Lysis Buffer

2.6.3.5.2 3µL of 1M DTT

2.6.3.5.3 7µL of Proteinase K

2.6.3.6 Screw the cap on the PrepFiler™ Bone and Tooth Lysate Tube, vortex it for 5 seconds, and then centrifuge it no longer than 5 seconds.

2.6.3.7 Incubate on a ThermoMixer® at 56°C and 1100 RPM for at least 2 hours (no more than 18 hours).

2.6.3.8 Centrifuge the tube for 90 seconds at ~10400 RPM.

2.6.3.9 Transfer the clear lysate (no sediment) to a new PrepFiler™ sample tube.

2.6.3.10 If the volume of the sample lysate collected in the sample tube is less than 150µL, add PrepFiler™ BTA Lysis Buffer to bring the lysate volume to 150µL.

2.6.3.11 Setup and run the AutoMate *Express*™ Forensic DNA Extraction System as described in step 2.6.2 above.

2.6.4 **Standard Extraction of DNA from Adhesive Sample Types Using PrepFiler *Express*™ BTA Kit (Lysate Preparation)**

2.6.4.1 Prepare the sample using standard laboratory procedures (reference CASE_4 (DNA Analysis)) or in consultation with the technical manager and/or Lead Analyst.

2.6.4.2 Preheat the ThermoMixer® to 56°C.

2.6.4.3 Place sample/substrate into PrepFiler™ Lysep Column/hinge-less sample tube assembly.

2.6.4.4 Add the following reagents to each sample:

2.6.4.4.1 220µL of PrepFiler™ BTA Lysis Buffer

2.6.4.4.2 3µL of 1M DTT

2.6.4.4.3 7µL of Proteinase K

2.6.4.5 Incubate on a ThermoMixer® at 56°C and 750 RPM for 40 minutes.

2.6.4.6 Centrifuge the column/tube assembly for 2 minutes at ~10400 RPM to transfer the lysate into the sample tube.

2.6.4.7 Remove the Lysep Column containing the substrate from the sample tube and retain, if necessary, or discard.

2.6.4.8 Transfer the clear lysate to a new PrepFiler sample tube.

2.6.4.9 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.

2.6.4.10 Setup and run the AutoMate *Express*™ Forensic DNA Extraction System as described in step 2.6.2 above.

2.7 **Notes**

2.7.1 Do not crosslink (UV) the PrepFiler™ Lysep Columns.

2.7.2 Do not use bleach on the AutoMate *Express*™ Forensic DNA Extraction System or racks (toxic fumes may be produced).

2.7.3 If the instrument door needs to be opened during a run, PAUSE the run before opening the door.

2.7.4 Steps taken to any samples must also be performed on an associated reagent blank per CWQM_9.5.1 (Analytical Procedures).

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_PrepFiler_Kits**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_ProK_DTT_Ext_Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **DNA_AutoMate_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Life Technologies™ PrepFiler *Express*™ and PrepFiler *Express* BTA™ Forensic DNA Extraction Kits User Guide

Eppendorf ThermoMixer® C Operating Manual

Life Technologies™ AutoMate *Express*™ Instrument User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_3 DIFFERENTIAL EXTRACTION & ISOLATION OF DNA (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)
Differential Extraction of DNA Using PrepFiler *Express*™ Kit (Lysate Preparation)
Isolation of DNA Using the AutoMate *Express*™ Forensic DNA Extraction System

3.1 Scope (Extraction Method)

Isolation of DNA from all samples testing presumptive or confirmatory positive for seminal fluid (sexual assault samples) using the PrepFiler *Express*™ Forensic DNA Extraction Kit and AutoMate *Express*™ Forensic DNA Extraction System.

3.2 Reagents & Supplies

3.2.1 1M dithiothreitol (DTT)

3.2.2 Proteinase K

3.2.3 E-cell Digest Buffer

3.2.4 Autoclaved water

3.2.5 PrepFiler *Express*™ Forensic DNA Extraction Kit

3.3 Equipment

3.3.1 Mechanical pipettors

3.3.2 Centrifuge

3.3.3 Vortex

3.3.4 Eppendorf ThermoMixer® C

3.3.5 AutoMate *Express*™ Forensic DNA Extraction System

3.4 Sample Preparation

Refer to CASE_4 (DNA Analysis) for guidance on sample types and appropriate extraction kit to use.

3.5 Controls

3.5.1 All extraction sets require the concurrent processing of at least one reagent blank.

3.5.2 Associated reagent blanks will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

3.5.3.1 1M dithiothreitol (DTT)

3.5.3.2 Proteinase K

3.5.3.3 E-cell Digest Buffer

3.5.3.4 Autoclaved water

3.5.3.5 PrepFiler *Express*™ Forensic DNA Extraction Kit

3.6 **Individual Steps of the Procedure**

3.6.1 **Differential Extraction of DNA Using PrepFiler *Express*™ Kit (Lysate Preparation)**

3.6.1.1 Preheat the ThermoMixer® to 56°C.

3.6.1.2 Place swab/substrate into a spin tube and add the following reagents to each sample:

3.6.1.2.1 395µL of E-cell Digest Buffer

3.6.1.2.2 5µL of Proteinase K

3.6.1.3 Vortex to mix and incubate on a ThermoMixer® at 56°C and 750 RPM for 1 hour.

3.6.1.4 After removing from ThermoMixer®, spin down samples briefly to eliminate condensation on tube caps.

3.6.1.5 Place swab/substrate in spin basket and spin at ~12,200 RPM for 5 minutes.

3.6.1.6 Save or discard swab/substrate.

3.6.1.7 Without disturbing the sperm pellet, transfer the supernatant (epithelial fraction) into a separate tube and process for DNA isolation as described in step 3.6.1.13 below. 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.

3.6.1.8 Wash the sperm pellet 3 times with autoclaved water as follows:

3.6.1.8.1 Add 500µL of autoclaved water to the sperm suspension.

3.6.1.8.2 Vortex and spin at ~12,200 RPM for 5 minutes.

3.6.1.8.3 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 supernatant can remain in the tube with the pellet after the final wash.

3.6.1.9 Add the following reagents to each sperm pellet from step 3.6.1.8.3:

3.6.1.9.1 500µL of PrepFiler™ Lysis Buffer

3.6.1.9.2 5µL of 1M DTT

3.6.1.10 (Optional Step) Add lysis/sperm pellet solution to a new tube to avoid evaporation during incubation.

3.6.1.11 Vortex, spin and incubate on a ThermoMixer® at 70°C and 750 RPM for 40 minutes.

3.6.1.12 Transfer the sperm lysate (~500µL) to a hinge-less sample tube labeled as sperm fraction.

3.6.1.13 For the epithelial fraction collected in step 3.6.1.7, perform the following:

3.6.1.13.1 Aliquot 50µL of epithelial fraction into a hinge-less sample tube.
NOTE: If more than 50µL of epithelial fraction is to be processed, based on case circumstances, concentrate the epithelial fraction, along with the associated reagent blank(s), to 50µL for use in order to maintain the desired salt concentration for binding DNA to magnetic particles.

3.6.1.13.2 To this aliquot, add 450µL of PrepFiler™ Lysis Buffer (without DTT because cells are already lysed) in order to bring the salt concentration to the desired level for DNA binding.

3.6.1.13.3 Proceed directly to automated extraction run.
NOTE: Both the epithelial fraction (after conditioning with PrepFiler™ Lysis Buffer) and sperm fraction can be processed simultaneously on the AutoMate *Express*™ Forensic DNA Extraction System (in one instrument run) since the protocol for isolation of DNA is identical for each fraction.

3.6.2 **Isolation of DNA Using the AutoMate *Express*™ Forensic DNA Extraction System**
Setup and run the AutoMate *Express*™ Forensic DNA Extraction System as follows:

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

3.6.2.2 Press start and open the door.

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

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

3.6.2.4.1 Row S (fourth row): Load with PrepFiler™ sample tubes containing lysate.

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

3.6.2.4.3 Row T1 (second row): Leave empty.

3.6.2.4.4 Row E (first row): Load with labeled PrepFiler™ elution tubes with caps open and secured.

3.6.2.5 Close the instrument door and press the enter arrow. Select the appropriate script (PrepFiler *Express*™ [1]).

3.6.2.6 Select 50uL elution volume, if applicable.

3.6.2.7 Press Start.

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

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

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

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

3.7 **Notes**

3.7.1 Do not crosslink (UV) the PrepFiler™ Lysep Columns.

3.7.2 Do not use bleach on the AutoMate *Express*™ Forensic DNA Extraction System or racks (toxic fumes may be produced).

3.7.3 If the instrument door needs to be opened during a run, PAUSE the run before opening the door.

3.7.4 Steps taken to any samples must also be performed on an associated reagent blank per CWQM_9.5.1 (Analytical Procedures).

3.7.5 The remaining epithelial fraction volume (and its associated reagent blank volume) should be stored in the refrigerator until the case is closed, in the event it is necessary to analyze it further.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_ProK_DTT_Ext_Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_E-Cell_Digest_Buffer**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Autoclaved_Water**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_PrepFiler_Kits**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **DNA_AutoMate_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Life Technologies™ PrepFiler *Express*™ and PrepFiler *Express* BTA™ Forensic DNA Extraction Kits User Guide

Eppendorf ThermoMixer® C Operating Manual

Life Technologies™ AutoMate *Express*™ Instrument User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_4 DNA QUANTIFICATION (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)
Preparing the Reaction
Running the Reaction
Analyzing the Results
Interpretation of Results

4.1 Scope

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

4.2 Reagents & Supplies

4.2.1 Quantifiler™ Trio DNA Quantification Kit

4.2.2 Quantifiler™ Automation Enhancer Kit

4.2.3 96-well optical reaction plate

4.2.4 Optical adhesive cover

4.2.5 Canned air (optional)

4.3 Equipment

4.3.1 Mechanical pipettors

4.3.2 Centrifuge

4.3.3 Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System

4.4 Reagent Preparation

4.4.1 Prepare the Quantifiler™ Working Lot by adding the Quantifiler™ Automation Enhancer Kit to the THP PCR Reaction Mix according to the Quantifiler™ Reagents OSBI Chemical Receipt/Function Verification Worksheet (**QCR_DNA_QF_Reagents**), if necessary.

4.4.2 Prepare the quantitation standards according to the Quantifiler™ Trio Quantitation Standards OSBI Chemical Formulation/Function Verification Worksheet (**QCR_DNA_Quant_Standards**), if necessary.

4.5 Controls

4.5.1 Each quantitation plate requires a Quantitation Negative Control (QNC)/Non-Template Control (NTC).

4.5.2 Associated reagent blank(s) will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

4.5.3.1 Quantifiler™ Working Lot

4.5.3.2 Quantifiler™ Trio DNA Quantification Kit

4.5.3.3 Quantifiler™ Automation Enhancer Kit

4.5.3.4 Quantifiler™ Trio Quantitation Standards

4.6 Individual Steps of the Procedure

4.6.1 Preparing the Reaction

4.6.1.1 Ensure the Quantifiler™ Trio Primer Mix and Quantifiler™ THP PCR Reaction Mix/Automation Enhancer mixture are thawed completely, vortex and spin down.

4.6.1.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Quantifiler™ Trio Primer Mix and Quantifiler™ THP PCR Reaction Mix/Automation Enhancer mixture to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Quantifiler™ Trio Primer Mix	8µL
Quantifiler™ THP PCR Reaction Mix/Automation Enhancer mixture	10µL

4.6.1.3 Dispense 18µL of Quantifiler™ Trio Master Mix into the appropriate wells of a 96-well reaction plate.

4.6.1.4 Pipette 2µL of each standard, sample and associated controls into the appropriate well.

4.6.1.5 Seal the plate with an optical adhesive cover ensuring that the seal is crosshatched for each well.

4.6.1.6 Spin down the plate until all air bubbles at the bottom of the wells are removed. Take care to ensure there are no bubbles in the plate before proceeding.

4.6.1.7 Ensure there are no particles or any dust on the plate. Individuals may use canned air to clean the plate.

4.6.2 Running the Reaction

4.6.2.1 Place the 96-well reaction plate in the QuantStudio™ 5 instrument and verify that it is seated correctly in the plate adapter before closing the instrument drawer.

4.6.2.2 Launch the HID Real-Time PCR Analysis Software v1.3 and log in using User Name: OSBI.

4.6.2.3 In the Home Screen, select the **Quantifiler™ Trio** icon.

4.6.2.4 In the Experimental Properties screen, enter an Experimental Name and User Name. All other settings on this screen are automatically set for the application or are optional.

4.6.2.5 Import the “Text (MS-DOS)” file from the appropriate Excel workbook attached to the policy manual or prepare the instrument as follows:

4.6.2.5.1 In the left navigation panel, select **Setup** > **Plate Setup**. The Quantifiler™ Trio targets (Large Autosomal, Small Autosomal, IPC and Y) are automatically specified for the application.

4.6.2.5.2 Define the samples: Select **Add New Sample**, then type the name of the sample and select the appropriate sample type (Standard, Unknown, NTC/QNC). Repeat for remaining samples. Alternatively, the samples may be imported into the HID software using an appropriate method.

4.6.2.5.3 Select the **Assign Targets and Samples** tab. To assign the samples to the plate wells, select the well and then click the **Assign** check box next to the appropriate sample. The targets and the standard quantities are automatically specified for each sample.

4.6.2.5.4 In the left navigation panel, select **Run** > **Run Method** to view the parameters. The parameters are automatically specified.

4.6.2.6 Save the file in the appropriate folder.

4.6.2.7 Click **Start Run** to begin the run.

4.6.3 **Analyzing the Results**

4.6.3.1 Verify the analysis settings by clicking **Analysis Settings** in the upper right corner of the window. Then click the **C_T Setting** tab and verify that the settings are correct. Then click **Apply Analysis Settings**.

Target	Threshold	Baseline Start	Baseline End
T. IPC	0.1	3	15
T. Large Autosomal	0.2	3	15
T. Small Autosomal	0.2	3	15
T. Y	0.2	3	15

4.6.3.2 Omit any unused wells by selecting only the unused wells, right click, and select **clear**.

4.6.3.3 Click **Analyze**.

4.6.3.4 To view the Standard Curve click **Analysis** in the left navigation pane, then click **Standard Curve** and select **All** in the Target drop-down list.

4.6.3.5 View the C_T values for the quantification standard reactions and then the calculated regression line slope, y-intercept, and R^2 values. The values should fall close to the same values for the lots of Quantifiler™ Trio kit and DNA quantitation standards used. If any values differ, analysts should be aware of the effects on the results of the quantitation.

4.6.3.5.1 Ensure the R^2 values are >0.98 for the small autosomal, large autosomal, and Y targets.

4.6.3.5.2 The “printer” icon on the top left side of the results screen may be used to print all standard curves onto one page at the same time.

4.6.3.6 Alternatively, the data may be exported to the appropriate Excel workbook attached to the policy manual for evaluation.

4.7 **Interpretation of Results**

4.7.1 The small autosomal target is used to estimate total human DNA concentration, while the Y target is used to estimate total human male DNA concentration. The large autosomal target is provided allow for a determination of any degradation.

4.7.2 The QNC should be "undet." during quantitation (no signal). If the QNC displays a signal, it will be marked as "Unknown" and reanalyzed to determine the amount of DNA detected. The technical manager and/or Lead Analyst shall be notified and determine if the plate is acceptable or if it will be re-setup and rerun.

4.7.3 Verify that all quantification standards performed properly.

4.7.4 Check samples and controls for possible inhibition or improper amplification including the IPC C_T for each sample. If observed, the sample may need to be re-quantitated.

IPC C_T flag triggered?	Degradation Index	Quality Index Interpretation†
No	<1	Typically indicates that DNA is not degraded or inhibited.
	1 to 10	Typically indicates that DNA is slightly to moderately degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.
	>10 or blank (no value)	Typically indicates that DNA is significantly degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.
Yes	<1	Although theoretically possible, this result is unlikely because PCR inhibitors in sufficient concentration to trigger the IPC C_T flag typically would affect the large autosomal target as well.
	>1 or blank (no value)	Typically indicates that the DNA is affected by degradation and/or PCR inhibition.

† These are general guidelines that may not apply to all samples depending on the inhibitors present, the varying quantity of contributor DNA in mixed samples, and the STR kit used.

4.7.5 Reagent blanks will be handled in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

4.7.6 If the quantitation value obtained for a sample is lower than desired or zero, the sample may be concentrated using a Vacufuge® plus in order to reach the desired amplification target; however, the same steps taken with the sample must also be done with at least one associated reagent blank per CWQM_9.5.1 (Analytical Procedures).

4.7.7 Any sample that has a low or zero DNA quantitation, which would require the full volume of the sample extract to be used for amplification, must have a total volume of extract not in excess of the associated reagent blank. Further concentration of any samples that have an

extract volume in excess of the associated reagent blank through additional process steps, such the use of Vacufuge® plus concentration, may be done at this point along with any associated reagent blanks.

4.7.8 Samples with inhibition should not be concentrated without consultation with the technical manager and/or Lead Analyst.

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

4.8 **Notes**

4.8.1 All reagents (primer mix, reaction mix, dilution buffer and DNA standard) should be stored at approximately -15 to -25°C upon receipt, and then approximately 2 to 8°C after initial use.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_QF_ Reagents**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Quant_Standards**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Quant_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Ext_Quant_Setup_Workbook_v1.0**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Life Technologies™ Quantifiler™ HP and Trio DNA Quantification Kits User Guide

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (for Human Identification) User Guide for use with: HID Real-Time PCR Analysis Software v1.3

Applied Biosystems™ HID Real-Time PCR Analysis Software Version 1.3 User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_5 DNA PURIFICATION & CONCENTRATION (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)
Purification of DNA Extracts Using the AutoMate *Express*™ Forensic DNA Extraction System
Concentration of DNA Extracts Using the Vacufuge® plus Concentrator

5.1 **Scope**
Purify and/or concentrate DNA extracts, as necessary.

5.2 **Reagents & Supplies**

5.2.1 PrepFiler *Express*™ or PrepFiler *Express* BTA™ Forensic DNA Extraction Kits

5.2.2 TE^{-4}

5.3 **Equipment**

5.3.1 Mechanical pipettors

5.3.2 AutoMate *Express*™ Forensic DNA Extraction System

5.3.3 Vacufuge® plus Concentrator

5.4 **Controls**

5.4.1 All purification and/or concentration steps must also be performed on an associated reagent blank per CWQM_9.5.1 (Analytical Procedures).

5.4.2 Requirement for lot numbers/expirations listed in case record (as applicable):

5.4.2.1 PrepFiler *Express*™ or PrepFiler *Express* BTA™ Forensic DNA Extraction Kits

5.4.2.2 TE^{-4}

5.5 **Individual Steps of the Procedure**

5.5.1 **Purification of DNA Extracts Using the AutoMate *Express*™ Forensic DNA Extraction System**
An additional AutoMate run may be performed to purify (clean) DNA extracts that have a colored/tinted appearance and/or detected inhibition after quantitation.

5.5.1.1 Transfer sample extract into a PrepFiler hinge-less sample tube, ensuring that at least one associated reagent blank undergoes the same concurrent processing.

5.5.1.2 Depending on sample type, add 500µL of either PrepFiler *Express*™ Lysis Buffer or PrepFiler™ BTA Lysis Buffer.

5.5.1.3 Setup and run the AutoMate *Express*™ Forensic DNA Extraction System as described in DNA_2.6.2.

5.5.2 **Concentration of DNA Extracts Using the Vacufuge® plus Concentrator**

5.5.2.1 With the Vacufuge® plus turned off and disconnected from the power supply, clean the device and the rotor prior to use (see Notes section below).

5.5.2.2 After the Vacufuge® plus is fully dry inside and out, insert the rotor. Connect to the power supply and turn it on.

5.5.2.3 Set the time to **15 minutes**, brake ***ON***, temperature to **60°C**, and mode/vent to ***V-AQ*** (vacuum – aqueous) and run the Vacufuge® plus (empty) to warm it up. (Note: The diaphragm vacuum pump (*vac*) indicator lamp lights up/switches to on during use at 1,000 rpm.)

5.5.2.4 Place the sample(s) and associated reagent blank(s) in the Vacufuge® plus with the lids of the tubes open, ensuring that the device is balanced, and set the time to unlimited (***oo***) or a desired time, brake ***ON***, temperature to **60°C**, and mode/vent set to ***V-AQ***.

5.5.2.5 Run the Vacufuge® plus for an amount of time required to concentrate (dry down) the sample/reagent blank volumes to an appropriate volume. In general, 20 minutes (or less) should be sufficient to bring samples to an appropriate volume. If a sample(s) require concentration for longer than 20 minutes, 5 minute (or less) increments should be used until an appropriate volume is achieved.

5.5.2.6 If necessary, add the required volume of TE^{-4} to bring the sample/reagent blank volume(s) back up to an appropriate volume for amplification.

5.5.2.7 Remove all samples/reagent blanks from the Vacufuge® plus and run it (empty) in mode/vent ***D-AQ*** (desiccator – aqueous) for 15 minutes (brake still ***ON*** and temperature at **60°C**) to remove the condensation from the pump and the hose system.

5.5.2.8 Turn off the Vacufuge® plus at the mains/power switch and disconnect the power plug from the power supply. Remove the rotor.

5.5.2.9 Empty the emission condenser, if necessary, and clean the Vacufuge® plus after use.

5.6 **Notes**

5.6.1 The purification and/or concentration of any sample and reagent blank must be documented in the case record.

5.6.2 The Vacufuge® plus rotor chamber is heated continuously, i.e. even when the rotor has stopped and the lid is open. It must be turned off after completing the application or the temperature set to **--** (no temperature).

5.6.3 The Vacufuge® plus should be thoroughly cleaned with ethanol or isopropyl alcohol. The rubber seals of the rotor chamber should be thoroughly cleaned with water and rubbed with glycerol or talcum powder, as necessary, to prevent from becoming brittle. Removing the rotor allows for easier cleaning.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_PrepFiler_Kits**

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_TE^{-4}**

OSBI Forensic Biology Units' Policy Manual **DNA_AutoMate_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Life Technologies™ PrepFiler *Express*™ and PrepFiler *Express* BTA™ Forensic DNA Extraction Kits User Guide

Life Technologies™ AutoMate *Express*™ Instrument User Guide

Eppendorf Concentrator plus/Vacufuge® plus Operating Manual

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_6 DNA AMPLIFICATION USING GLOBALFILER™ (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)

6.1 Scope

STR amplification of DNA samples using the GlobalFiler™ PCR Amplification Kit.

6.2 Reagents & Supplies

6.2.1 GlobalFiler™ PCR Amplification Kit

6.2.2 TE^{-4}

6.2.3 PCR tubes (0.2mL GeneAmp™) or 96-well optical reaction plate

6.2.4 PCR plate foil (if applicable)

6.3 Equipment

6.3.1 Mechanical pipettors

6.3.2 Vortex

6.3.3 Centrifuge or MiniSpin

6.3.4 Applied Biosystems™ ProFlex™ PCR System (thermal cycler)

6.4 Controls

6.4.1 Positive control: 10µL control DNA (0.1ng/µL) and 5µL of TE^{-4}, or alternatively, less control DNA and more TE^{-4} may be used if excessive pull-up occurs with a particular GlobalFiler™ kit lot. For example, 7µL of control DNA and 8µL of TE^{-4} can be used.

6.4.2 Negative control: 15µL of TE^{-4}.

6.4.3 Associated reagent blank(s) and controls will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

6.4.4.1 GlobalFiler™ PCR Amplification Kit

6.4.4.2 TE^{-4}

6.5 Individual Steps of the Procedure

6.5.1 Vortex the GlobalFiler™ Master Mix and Primer Mix and spin down.

6.5.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix and Primer Mix to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
GlobalFiler™ Master Mix	7.5µL
GlobalFiler™ Primer Mix	2.5µL

6.5.3 Dispense 10µL of PCR master mix into each tube/well.

6.5.4 Add the appropriate volume of sample and/or TE^{-4} to each tube/well to reach a total input volume of 15µL. A target range of 0.5 to 1.0ng based on sample type/quantitation/analyst discretion is recommended. If the target range of 0.5 to 1.0ng cannot be reached, based on a low quantity of DNA present in the sample, amplification of the maximum volume (15µL) of sample will be performed.

6.5.5 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

6.5.6 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for GlobalFiler™ per the 29 cycle internal validation.

6.5.7 Remove the tubes/plate from the thermal cycler after the amplification is complete.

6.6 **Notes**

6.6.1 Amplified products shall be stored refrigerated or frozen and must be protected from light.

6.6.2 Amplified products may not be used after two weeks from the date of amplification without technical manager approval.

6.6.3 Upon completion of the technical review of the case or batch, amplified products should be discarded.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Amp_Kit**

OSBI Forensic Biology Units' Policy Manual **DNA_Normalization** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_GF_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_YFP_Amp_Plate_Maps**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Applied Biosystems™ GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide

Life Technologies™ ProFlex™ PCR System User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_7 DNA AMPLIFICATION USING YFILER™ PLUS (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)

7.1 Scope

Y-STR amplification of DNA samples for male component using the Yfiler™ Plus PCR Amplification Kit.

7.2 Reagents & Supplies

7.2.1 Yfiler™ Plus PCR Amplification Kit

7.2.2 TE^{-4}

7.2.3 PCR tubes (0.2mL GeneAmp™) or 96-well optical reaction plate

7.2.4 PCR plate foil (if applicable)

7.3 Equipment

7.3.1 Mechanical pipettors

7.3.2 Vortex

7.3.3 Centrifuge or MiniSpin

7.3.4 Applied Biosystems™ ProFlex™ PCR System (thermal cycler)

7.4 Controls

7.4.1 Positive control: Prepare a 1:20 dilution of the provided control DNA (2.0ng/µL) and use 10µL for amplification (e.g. up to 10µL of 2.0ng/µL 007 standard diluted in 190µL TE^{-4}). Less control DNA and more TE^{-4} may be used if excessive pull-up occurs with a particular Yfiler™ Plus kit lot. For example, 9µL of diluted control DNA and 1µL of TE^{-4} can be used.

7.4.2 Negative control: 10µL of TE^{-4}.

7.4.3 Associated reagent blank(s) and controls will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

7.4.4.1 Yfiler™ Plus PCR Amplification Kit

7.4.4.2 TE^{-4}

7.5 Individual Steps of the Procedure

7.5.1 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

7.5.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix and Primer Set to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL

7.5.3 Dispense 15µL of PCR master mix into each tube/well.

7.5.4 Add the appropriate volume of sample and/or TE^{-4} to each tube/well to reach a total input volume of 10µL. A target range of 0.5 to 1.0ng based on sample type/quantitation/analyst discretion is recommended. If the target range of 0.5 to 1.0ng cannot be reached, based on a low quantity of DNA present in the sample, amplification of the maximum volume (10µL) of sample will be performed.

7.5.5 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

7.5.6 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus per the 30 cycle internal validation.

7.5.7 Remove the tubes/plate from the thermal cycler after the amplification is complete.

7.6 **Notes**

7.6.1 Amplified products shall be stored refrigerated or frozen and must be protected from light.

7.6.2 Amplified products may not be used after two weeks from the date of amplification without technical manager approval.

7.6.3 Upon completion of the technical review of the case, amplified products should be discarded.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Amp_Kit**

OSBI Forensic Biology Units' Policy Manual **DNA_Normalization** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_YFP_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_YFP_Amp_Plate_Maps**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Applied Biosystems™ Yfiler™ Plus PCR Amplification Kit User Guide

Life Technologies™ ProFlex™ PCR System User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_8 DNA AMPLIFICATION USING GLOBALFILER™ EXPRESS (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)

8.1 **Scope**
STR amplification of DNA samples using the GlobalFiler™ Express PCR Amplification Kit.

8.2 **Reagents & Supplies**

8.2.1 GlobalFiler™ Express PCR Amplification Kit

8.2.2 Prep N Go Lysis Buffer

8.2.3 TE^{-4}

8.2.4 PCR tubes (0.2mL GeneAmp™) or 96-well optical reaction plate

8.2.5 PCR plate foil (if applicable)

8.3 **Equipment**

8.3.1 Mechanical pipettors

8.3.2 Sample punch tool

8.3.3 Vortex

8.3.4 Centrifuge or MiniSpin

8.3.5 Eppendorf ThermoMixer®

8.3.6 Applied Biosystems™ ProFlex™ PCR System (thermal cycler)

8.4 **Controls**

8.4.1 Positive control: 3µL control DNA (2.0ng/µL)

8.4.2 Negative control: See below

8.4.3 Associated controls will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

8.4.4.1 GlobalFiler™ Express PCR Amplification Kit

8.4.4.2 Prep N Go Lysis Buffer

8.4.4.3 TE^{-4}

8.5 **Individual Steps of the Procedure**

8.5.1 If necessary, prepare the GlobalFiler™ Express kit by adding the following volume of Master Mix Additive to the GlobalFiler™ Express Master Mix tube(s):

Kit	*Master Mix Additive Volume*
GFE 200 reactions	80uL
GFE 1000 reactions	390uL

Gently invert the Master Mix tube ~10 times, then centrifuge briefly. Mark the cap of the Master Mix tube with a (+) to indicate that the Additive has been added. Discard the Additive tube.

8.5.2 Gently invert the Master Mix tube approximately 10 times, then centrifuge briefly.

8.5.3 Vortex Primer Mix and spin down.

8.5.4 Prepare a PCR Reaction Mix for all samples to be amplified by adding the appropriate volumes of Master Mix and Primer Mix to a tube, vortex and spin down.

Reaction Mix Preparation	Volume/Sample
GlobalFiler™ Express Master Mix	6µL
GlobalFiler™ Express Primer Mix	6µL

8.5.5 **Prepare Samples:**

8.5.5.1 **For Buccal Cells on FTA Card, Bloodstains of FTA Card:**

8.5.5.1.1 Punch 1.2mm disc(s) from buccal cell or bloodstain samples into 0.2mL tube or 96-well plate, with two cleaning strikes between punches

8.5.5.1.2 Add 3uL TE^{-4} (per sample) to the Reaction Mix

8.5.5.1.3 Dispense 15uL of the Reaction Mix to each tube or well containing a sample

8.5.5.2 **For Bloodstains on Non-FTA Card:**

8.5.5.2.1 Punch 1.2mm disc(s) from buccal cell or bloodstain samples into 0.2mL tube or 96-well plate, with two cleaning strikes between punches

8.5.5.2.2 Add 3uL Prep N Go Lysis Buffer (per sample) to the Reaction Mix

8.5.5.2.3 Dispense 15uL of the Reaction Mix to each tube or well containing a sample

8.5.5.3 **For Buccal Cells on Cotton Swab or Buccal Cells on Foam-Tip Swab:**

8.5.5.3.1 Cut approximately ¼ of foam tip or cotton head and place into 1.5mL tube

8.5.5.3.2 Add 400uL Prep N Go Lysis Buffer to each sample and reagent blank tube

8.5.5.3.3 Incubate on ThermoMixer for 20 minutes at 90 degrees, shaking at 750 RPM; ThermoMixer lid is to remain off during this incubation

8.5.5.3.4 Dispense 12uL of the Reaction Mix to each tube or well containing a sample or reagent blank

8.5.5.3.5 Dispense 3uL of each sample and reagent blank lysate into each tube or well

8.5.5.4 **For Bloodstain on Cloth:**

8.5.5.4.1 Place one ~0.5cm x 0.5cm cutting from bloodstained cloth into 1.5mL tube

8.5.5.4.2 Add 200uL Prep N Go Lysis Buffer to each sample and reagent blank tube

8.5.5.4.3 Incubate on ThermoMixer for 20 minutes at 90 degrees, shaking at 750 RPM; ThermoMixer lid is to remain off during this incubation

8.5.5.4.4 Dispense 12uL of the Reaction Mix to each tube or well containing a sample or reagent blank

8.5.5.4.5 Dispense 3uL of each sample and reagent blank lysate into each tube or well

8.5.6 **Prepare Amplification Controls:**

8.5.6.1 **Negative Control:**

8.5.6.1.1 For Buccal Cells on FTA Card, Bloodstains of FTA Card: 12uL Reaction Mix and 3uL TE^{-4}.

8.5.6.1.2 For Bloodstains on Non-FTA Card, Buccal Cells on Cotton Swab, Buccal Cells on Foam-Tip Swab, Bloodstain on cloth: 12uL Reaction Mix plus 3uL Prep N Go Lysis Buffer

8.5.6.1.3 Do not add blank substrate disc to a negative amplification control

8.5.6.2 **Positive Control:** 12uL Reaction Mix plus 3uL Control DNA 007

8.5.7 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

8.5.8 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for GlobalFiler™ Express per the 27 cycle internal validation.

8.5.9 Remove the tubes/plate from the thermal cycler after the amplification is complete.

8.6 **Notes**

8.6.1 When taking punches from FTA or non-FTA cards, take care not to punch through the backing material, as the paper backing can cause inhibition during amplification.

8.6.2 Sample and reagent blank lysates may only be utilized for direct amplification on the same day they are created.

8.6.3 Amplified products shall be stored refrigerated or frozen and must be protected from light.

8.6.4 Amplified products may not be used after two weeks from the date of amplification without technical manager approval.

8.6.5 Upon completion of the technical review of the case or batch, amplified products should be discarded.

Attachment(s):

OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Amp_Kit**

OSBI Forensic Biology Units' Policy Manual **DNA_Normalization** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_GF_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_YFP_Amp_Plate_Maps**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):

Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kits User Guide

Life Technologies™ ProFlex™ PCR System User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_9 DNA AMPLIFICATION USING YFILER™ PLUS in Direct Mode (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)

9.1 **Scope**
Y-STR amplification of DNA samples for male component using the Yfiler™ Plus PCR Amplification Kit in Direct Mode.

9.2 **Reagents & Supplies**

9.2.1 Yfiler™ Plus PCR Amplification Kit

9.2.2 Prep-N-Go Lysis Buffer

9.2.3 TE^{-4}

9.2.4 PCR tubes (0.2mL GeneAmp™) or 96-well optical reaction plate

9.2.5 PCR plate foil (if applicable)

9.3 **Equipment**

9.3.1 Mechanical pipettors

9.3.2 Sample punch tool

9.3.3 Vortex

9.3.4 Centrifuge or MiniSpin

9.3.5 Eppendorf ThermoMixer

9.3.6 Applied Biosystems™ ProFlex™ PCR System (thermal cycler)

9.4 **Controls**

9.4.1 Positive control: Use 2.0ng/µL 007 standard and indicated below.

9.4.2 Negative control: See below

9.4.3 Associated controls will be setup and evaluated in accordance with CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

9.4.4.1 Yfiler™ Plus PCR Amplification Kit

9.4.4.2 Prep-N-Go Lysis Buffer

9.4.4.3 TE^{-4}

9.5 Individual Steps of the Procedure

9.5.1 For Buccal Cells on Cotton Swab and Buccal Cells on Foam-tip Swabs:

9.5.1.1 For each swab sample, remove approximately ¼ of the cotton or foam-tip swab head and place into a 1.5mL tube. Add 400uL of Prep-N-Go Buffer to each sample and associated reagent blank tube, and incubate on ThermoMixer for 20 minutes at 90*C, shaking at 750 RPM (lid of ThermoMixer removed during incubation).

9.5.1.2 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

9.5.1.3 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix, Primer Set, and TE^{-4} to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL
TE^{-4}	10uL

9.5.1.4 Dispense 25µL of PCR master mix into each tube/well.

For each sample and reagent blank, add 3uL of lysate. For Positive Control, add 3uL of Control DNA 007 (2ng/uL). For Negative Control, add 3uL of Prep-N-Go Buffer.

9.5.1.5 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

9.5.1.6 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus Direct Mode per the 28 cycle internal validation.

9.5.1.7 Remove the tubes/plate from the thermal cycler after the amplification is complete.

9.5.2 For Buccal Cells on FTA card:

9.5.2.1 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

9.5.2.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix, Primer Set, and TE^{-4} to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL
TE^{-4}	12uL

9.5.2.3 Using sample punch tool, punch 1.2mm discs from buccal cells on FTA card into tube/plate well for each sample, with two cleaning strikes between punches.

9.5.2.4 Dispense 27µL of PCR master mix into each tube/well.

9.5.2.5 For Positive Control, add 3uL of Control DNA 007 (2ng/uL). For Negative Control, no additional reagent(s) added (Master Mix only).

9.5.2.6 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.
9.5.2.7 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus Direct Mode per the 28 cycle internal validation.

9.5.3 **For Bloodstains on Cloth:**

9.5.3.1 For each sample, place one ~0.5cm x 0.5cm cutting from bloodstained cloth into a 1.5mL tube. Add 200uL of Prep-N-Go Buffer to each sample and associated reagent blank tube, and incubate on ThermoMixer for 20 minutes at 90*C, shaking at 750 RPM (lid of ThermoMixer removed during incubation).

9.5.3.2 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

9.5.3.3 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix, Primer Set, and TE^{-4} to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL
TE^{-4}	10uL

9.5.3.4 Dispense 25µL of PCR master mix into each tube/well.

9.5.3.5 For each sample and reagent blank,, add 3uL of lysate. For Positive Control, add 3uL of Control DNA 007 (2ng/uL). For Negative Control, add 3uL of Prep-N-Go Buffer.

9.5.3.6 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

9.5.3.7 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus Direct Mode per the 28 cycle internal validation.

9.5.4 **For Bloodstains on FTA Card:**

9.5.4.1 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

9.5.4.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix, Primer Set, and TE^{-4} to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL
TE^{-4}	12uL

9.5.4.3 Using sample punch tool, punch 1.2mm discs from bloodstain on FTA card into tube/plate well for each sample, with two cleaning strikes between punches.

9.5.4.4 Dispense 27µL of PCR master mix into each tube/well.

9.5.4.5 For Positive Control, add 3uL of Control DNA 007 (2ng/uL). For Negative Control, no additional reagent(s) added (Master Mix only).

9.5.4.6 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

9.5.4.7 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus Direct Mode per the 28 cycle internal validation.

9.5.5 **For Bloodstain on non-FTA Card:**

9.5.5.1 Vortex the Yfiler™ Plus Master Mix and Primer Set and spin down.

9.5.5.2 Prepare a PCR master mix for all samples to be amplified by adding the appropriate volumes of Master Mix, Primer Set, Prep N Go buffer, and TE^{-4} to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Yfiler™ Plus Master Mix	10µL
Yfiler™ Plus Primer Set	5µL
TE^{-4}	10uL
Prep-N-Go Buffer	2uL

9.5.5.3 Using sample punch tool, punch 1.2mm discs from bloodstain on FTA card into tube/plate well for each sample, with two cleaning strikes between punches.

9.5.5.4 Dispense 27µL of PCR master mix into each tube/well.

9.5.5.5 For Positive Control, add 2uL of Control DNA 007 (2ng/uL). For Negative Control, no additional reagent(s) added (Master Mix only).

9.5.5.6 Cap the tubes/seal the plate with PCR foil and spin down the tubes/plate.

9.5.5.7 Place the tubes/plate in the ProFlex™ PCR System (thermal cycler) with the provided compression pad on top of the tubes/plate (brown side to the top) and start the amplification program for Yfiler™ Plus Direct Mode per the 28 cycle internal validation

9.6 **Notes**

9.6.1 When taking punches from FTA or non-FTA cards, take care not to punch through the backing material, as the paper backing can cause inhibition during amplification.

9.6.2 Sample and reagent blank lysates may only be utilized for direct amplification on the same day they are created.

9.6.3 Amplified products shall be stored refrigerated or frozen and must be protected from light.

9.6.4 Amplified products may not be used after two weeks from the date of amplification without technical manager approval.

9.6.5 Upon completion of the technical review of the case, amplified products should be discarded.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Amp_Kit**

OSBI Forensic Biology Units' Policy Manual **DNA_Normalization** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_YFP_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_YFP_Amp_Plate_Maps**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Applied Biosystems™ Yfiler™ Plus PCR Amplification Kit User Guide

Life Technologies™ ProFlex™ PCR System User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_10 3500 SERIES GENETIC ANALYZER ANALYSIS (↑ Table of Contents)

Quick links:
CWQM_9 (Analytical Procedures)
CASE_4 (DNA Analysis)
Preparing Samples, Controls & Ladders for the 3500 Series Genetic Analyzer
Loading Samples onto the 3500 Series Genetic Analyzer
Electrophoresis of Samples Using the 3500 Series Genetic Analyzer

10.1 Scope

Obtaining a genetic profile using capillary electrophoresis and the 3500 Series Genetic Analyzer from DNA samples previously amplified with the GlobalFiler™, Yfiler™ Plus, GlobalFiler™ Express, and/or Yfiler™ Plus in Direct Mode PCR Amplification Kit.

10.2 Reagents & Supplies

10.2.1 96-well optical reaction plate

10.2.2 96-well septa mat

10.2.3 Plate base and retainer

10.2.4 Microcentrifuge tube(s)

10.2.5 GlobalFiler™, Yfiler™ Plus, and/or GlobalFiler™ Express Allelic Ladder

10.2.6 Hi-Di™ Formamide or equivalent

10.2.7 GeneScan™ 600 LIZ™ Size Standard v2.0 (GS600 LIZ™ v2.0)

10.2.8 3500 Series Genetic Analyzer consumables: capillary array, Performance Optimized Polymer 4 (POP-4™), and Anode/Cathode Buffer Containers (ABC/CBC)

10.3 Equipment

10.3.1 Mechanical pipettors

10.3.2 Vortex

10.3.3 MiniSpin

10.3.4 Centrifuge

10.3.5 Available equipment for performing heat denature/snap cool (e.g. thermal cycler/freezer, ice or cold block

10.3.6 Applied Biosystems™ 3500 Series Genetic Analyzer

10.4 Controls

10.4.1 Run reagent blanks according to the most sensitive injection conditions per CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

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

10.4.2.1 Hi-Di™ Formamide

10.4.2.2 GeneScan™ 600 LIZ™ Size Standard v2.0

10.4.2.3 3500 Performance Optimized Polymer 4

10.4.2.4 3500 Anode/Cathode Buffer Containers

10.5 Individual Steps of the Procedure

10.5.1 Preparing Samples, Controls & Ladders for the 3500 Series Genetic Analyzer

10.5.1.1 Prepare a master mix for all samples to be ran (GlobalFiler™, Yfiler™ Plus, GlobalFiler™ Express, and/or Yfiler™ Plus in Direct Mode) by adding the appropriate volumes of Hi-Di™ Formamide and GS600 LIZ™ v2.0 to a tube, vortex and spin down.

Master Mix Preparation	Volume/Sample
Hi-Di™ Formamide	9.6µL
GS600 LIZ™ v2.0	0.4µL

10.5.1.2 Aliquot 10µL of master mix into each well with a sample.

10.5.1.3 Aliquot 10µL of master mix or Hi-Di™ Formamide only into empty wells in each column containing samples in an injection set.

10.5.1.4 Add 1µL of sample/control/ladder to each well.

10.5.1.5 Seal plate with septa mat and spin down.

10.5.1.6 Heat denature each sample at ~95°C for ~3 minutes.

10.5.1.7 Snap cool each sample at ~0°C for ~3 minutes.

10.5.2 Loading the 3500 Series Genetic Analyzer

10.5.2.1 Snap the plate retainer over the sample plate and plate base.

10.5.2.2 Place the assembly onto the auto sampler.

10.5.3 Capillary Electrophoresis Using the 3500 Series Genetic Analyzer

10.5.3.1 Open the **3500 Series** software (Data Collection Software) and wait for the service console to start all application services.

10.5.3.2 After the Dashboard screen launches, press the “Refresh” button to ensure the most up-to-date information is displayed for the instrument.

10.5.3.3 (Optional) Preheat the oven to 60°C from the main dashboard.

10.5.3.4 Click **Create New Plate** in the Dashboard.

10.5.3.5 In the *Define Properties* screen, enter the plate name for the plate and ensure that **96** is selected for the Number of Wells, **36cm** is selected for Capillary Length, **HID** is

selected for Plate Type, and **POP4** is selected for the Polymer Type. The plate can be saved at this point, if desired.

10.5.3.6 Click **Assign Plate Contents** and fill in the appropriate Sample Names, Sample Types, Assay, Filename Convention, and Results Group (the plate view or table view can be used to enter this information).

10.5.3.6.1 Assays:

10.5.3.6.1.1 GlobalFiler and Yfiler Plus:

- 7s_Globalfiler_Yfiler_Plus
- 15s_Globalfiler_Yfiler_Plus
- 24s_Globalfiler_Yfiler_Plus

10.5.3.6.1.2 GlobalFiler Express and Yfiler Plus Direct Mode:

- OSR_7s_Globalfiler_Express_Yfiler_Plus
- OSR_15s_Globalfiler_Express_Yfiler_Plus
- OSR_24s_Globalfiler_Express_Yfiler_Plus

10.5.3.6.2 Sample names must reflect the biologist's initials, case number, item number, and kit type.

10.5.3.6.3 Controls must reflect the biologist's initials, case number and/or date, control name, and kit type.

10.5.3.6.4 Ladders must reflect "Ladder" and kit type.

10.5.3.6.5 Do not use special characters (other than an underscore) as they are not recognized by the software and can cause the software to be unable to find the plate records in the datastore.

10.5.3.7 Another option is to import a plate from a template. To do this, after you click on **Create New Plate**, enter the plate name and click on **Assign Plate Contents**. In the next screen select the **Import** button and import the plate from the appropriate location. Then assign and/or verify the Sample Types, Assays, File Name Convention, and Results Group, as appropriate.

10.5.3.8 At this point, either click **View Plate Grid Report** and print this view for the case file or use the 3500 setup worksheet for the case file. If using the 3500 Plate Grid Report, adjust the view of the table before printing by zooming out, as needed; the view seen on the screen is what prints.

10.5.3.9 Save the plate and click on **Link Plate for Run**.

10.5.3.10 Click on **Create Injection List**. From here, duplicate injections may be setup, as necessary. Any reinjection that needs to be performed can be designated from this screen only while the run is in progress. Alternatively, the injection list may be modified before the run is started in the *Preview Run* screen. To duplicate an injection (same parameters), select an injection and then click the icon with two downward pointing arrows. To use a different protocol for a replicate injection, specify a reinjection in the *Monitor Run* screen after you start the run.

10.5.3.11 Click on the **Start Run** button.

10.6 **Notes**

10.6.1 It is recommended a ladder be run every 24 samples.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QCR_DNA_Formamide**

OSBI Forensic Biology Units' Policy Manual **DNA_3500_Setup** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

Reference(s):
Applied Biosystems™ 3500/3500xL Genetic Analyzer User Guide

Applied Biosystems™ DNA Fragment Analysis by Capillary Electrophoresis User Guide

Applied Biosystems™ Data Collection Software 4.0 User Bulletin (Rev D)

Applied Biosystems™ GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits User Guide

Applied Biosystems™ Yfiler™ Plus PCR Amplification Kit User Guide

Applied Biosystems™ GlobalFiler Express™ PCR Amplification Kit User Guide

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

DNA_11 GENEMAPPER® *ID-X* DATA ANALYSIS (↑ Table of Contents)

11.1 Scope

The GeneMapper® *ID-X* (GMID-X) Software analyzes the data collected by the Genetic Analyzer to size and quantify the DNA fragments.

11.2 Equipment

11.2.1 Computer with GeneMapper® *ID-X* Software Version 1.6

11.3 Individual Steps of the Procedure

11.3.1 Launch the **GeneMapper ID-X** application.

11.3.2 Add sample files to the Project Window.

11.3.3 Select the appropriate Sample Type.

11.3.3.1 Allelic ladders as “Allelic Ladder”

11.3.3.2 Forensic samples as “Sample”

11.3.3.3 Reagent blanks and negative controls as “Negative Control”

11.3.3.4 Positive control as “Positive Control”

11.3.4 Select the appropriate Analysis Method.

11.3.4.1 **GF_7s_15s_OSBI_Casework** for analysis of GlobalFiler™ 7 second and 15 second injections. The parameters should be defined as follows:

11.3.4.1.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- “Use marker-specific stutter ratio if available” selected
- Global Minus Stutter Distance for Tri From: 2.25 To: 3.75
- Global Minus Stutter Distance for Tetra From: 3.25 To: 4.75
- All other values should be 0.0

11.3.4.1.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 65 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

GlobalFiler™ Analytical Thresholds	
Dye Channel	**7, 15, 24 seconds**
Blue	100
Green	100
Yellow	100
Red	100
Purple	100

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts
- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

11.3.4.1.3 Peak Quality Tab

- Homozygous Minimum Peak Height: 530.0
- Max Peak Height (MPH): 30000
- Min. Peak Height Ratio: 0.6

11.3.4.2 **GF_24s_OSBI_Casework** for analysis of GlobalFiler™ 24 second injections. The parameters should be defined as follows:

11.3.4.2.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- "Use marker-specific stutter ratio if available" selected
- Global Minus Stutter Distance for Tri From: 2.25 To: 3.75
- Global Minus Stutter Distance for Tetra From: 3.25 To: 4.75
- All other values should be 0.0

11.3.4.2.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 65 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

GlobalFiler™ Analytical Thresholds	
Dye Channel	**7, 15, 24 seconds**
Blue	100
Green	100
Yellow	100
Red	100
Purple	100

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts
- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

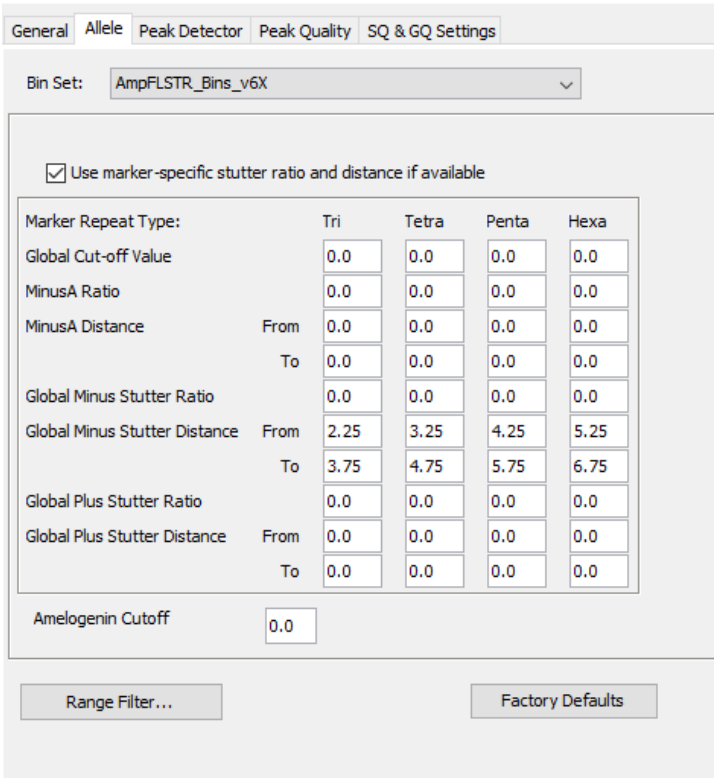
11.3.4.2.3 Peak Quality Tab

- Homozygous Minimum Peak Height: 820.0
- Max Peak Height (MPH): 30000
- Min. Peak Height Ratio: 0.6

11.3.4.3 **YFP_7s_15s_OSBI_Casework** for analysis of Yfiler™ Plus 7 second and 15 second injections. The parameters should be defined as follows:

11.3.4.3.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- "Use marker-specific stutter ratio if available" selected
- Use stutter range settings for markers of **all sizes** as shown:



11.3.4.3.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 60 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

Yfiler™ Plus Analytical Thresholds	
Dye Channel	**7 & 15 seconds**
Blue	100
Green	100
Yellow	100
Red	100
Purple	100

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts
- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

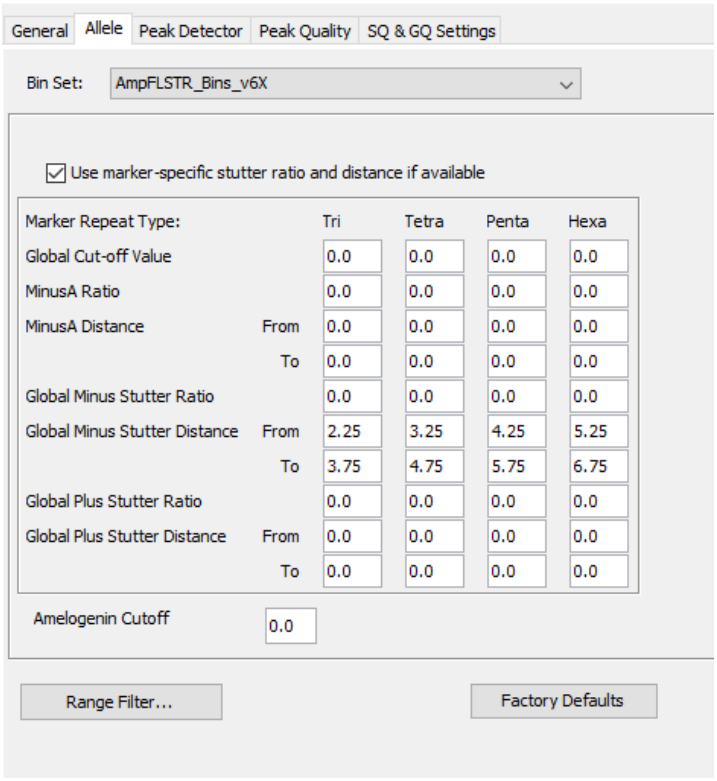
11.3.4.3.3 Peak Quality tab:

- Homozygous Minimum Peak Height: 300.0
- Max Peak Height (MPH): 30000
- Min. Peak Height Ratio: 0.6

11.3.4.4 **YFP_24s_OSBI_Casework** for analysis of Yfiler™ Plus 24 second injections. The parameters should be defined as follows:

11.3.4.4.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- "Use marker-specific stutter ratio if available" selected
- Use stutter range settings for markers of **all sizes** as shown:



11.3.4.4.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 60 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

Yfiler™ Plus Analytical Thresholds	
Dye Channel	**24 seconds**
Blue	150
Green	150
Yellow	150
Red	150
Purple	150

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts
- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

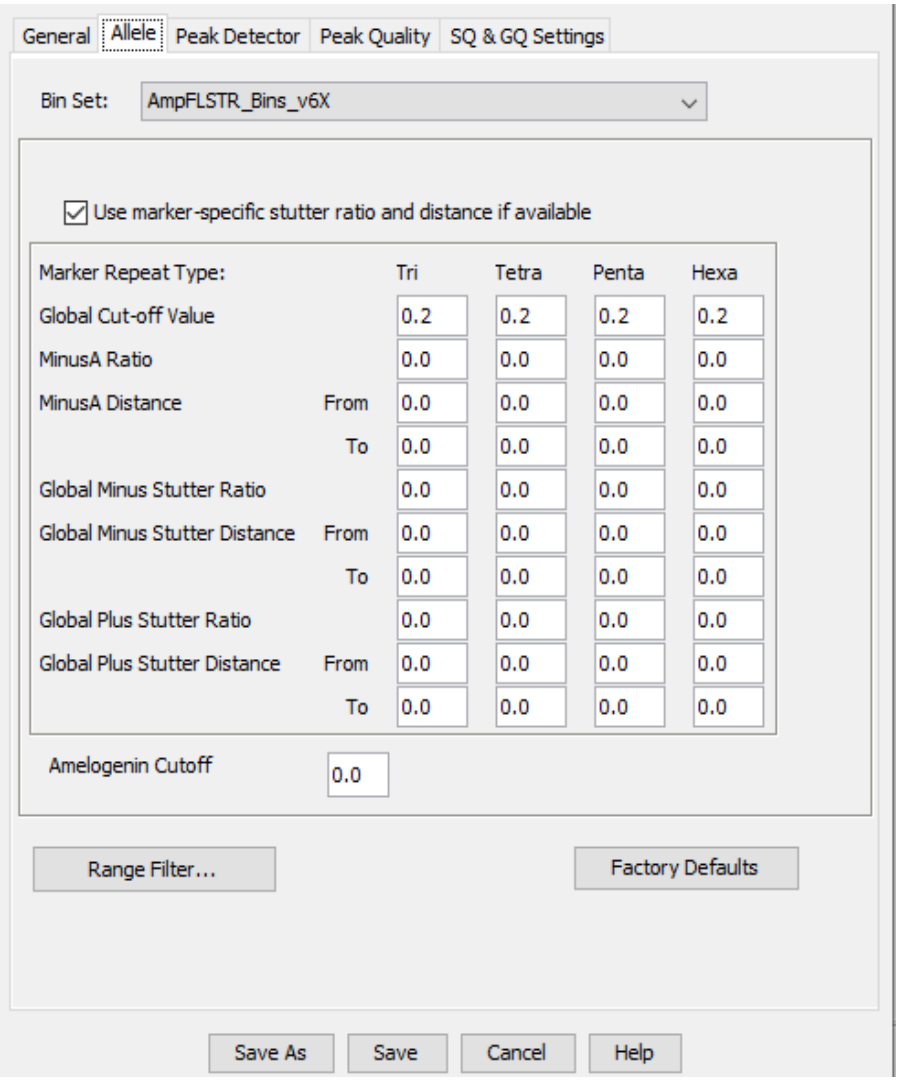
11.3.4.4.3 Peak Quality tab:

- Homozygous Minimum Peak Height: 400.0
- Max Peak Height (MPH): 30000
- Min. Peak Height Ratio: 0.6

11.3.4.5 **GFE_OSBI_Casework** for analysis of GlobalFiler™ Express 7 second ,15 second, and 24 second injections. The parameters should be defined as follows:

11.3.4.5.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- "Use marker-specific stutter ratio if available" selected 20% Global Filter
- Use stutter range settings for markers of **all sizes** as shown:



11.3.4.5.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 65 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

GlobalFiler™ Express Analytical Thresholds	
Dye Channel	**7, 15, 24 seconds**
Blue	120
Green	120
Yellow	120
Red	120
Purple	120

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts
- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

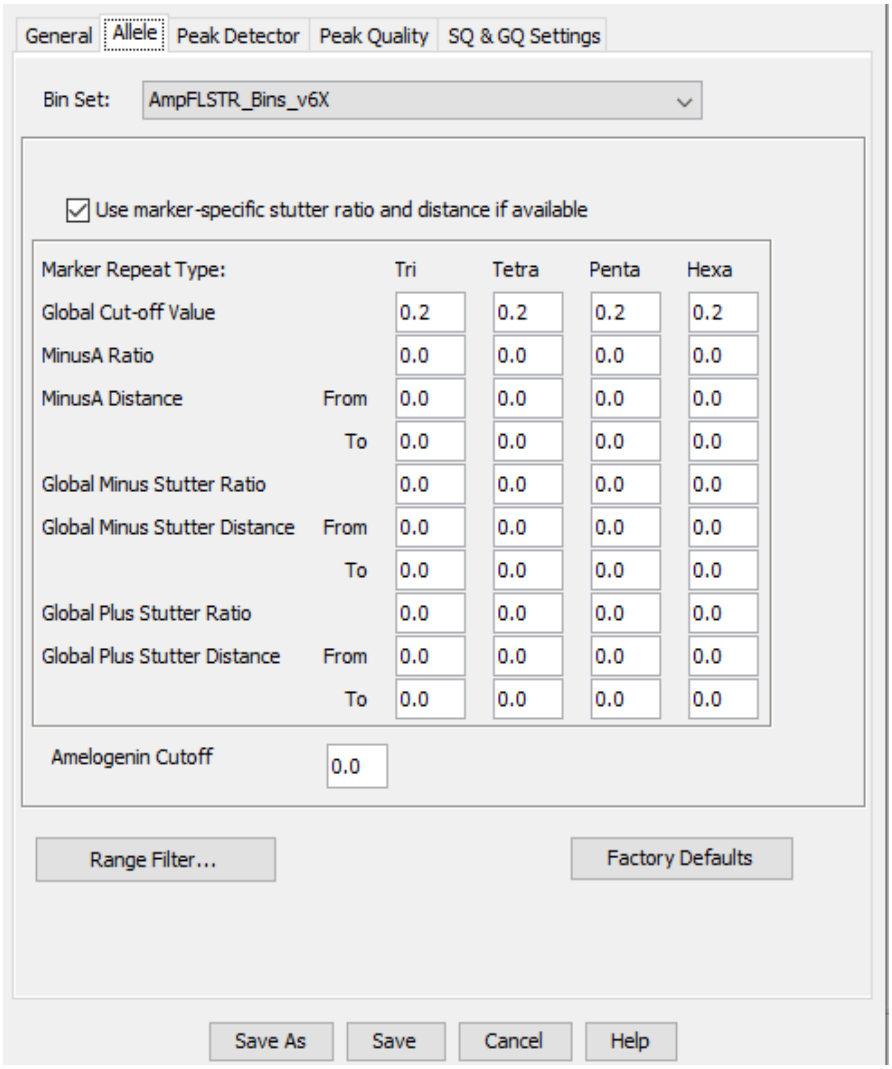
11.3.4.5.3 Peak Quality tab:

- Max Peak Height (MPH): 50,000RFU
- Homozygous Minimum Peak Height: 250.0
- Min. Peak Height Ratio: 0.6

11.3.4.6 **YFP_Direct_OSBI_Casework** for analysis of Yfiler™ Plus in Direct Mode 7 second ,15 second, and 24 second injections. The parameters should be defined as follows:

11.3.4.6.1 Allele Tab:

- Bin Set: AmpFLSTR_BINS_v6x
- “Use marker-specific stutter ratio if available” selected20% Global Filter
- Use stutter range settings for markers of **all sizes** as shown:



11.3.4.6.2 Peak Detector Tab:

- Peak Detection Algorithm: Advanced
- Sizing: Partial Sizes Start Size: 60 Stop Size: 460
- Smoothing: Light
- Baseline Window: 33 pts
- Size Calling Method: Local Southern Method
- Peak Amplitude Thresholds:

Yfiler™ Plus Direct Analytical Thresholds	
Dye Channel	**7, 15, 24 seconds**
Blue	120
Green	120
Yellow	120
Red	120
Purple	120

- Min. Peak Half Width: 2 pts.
- Polynomial Degree: 3
- Peak Window Size: 13 pts

- Peak Start: 0.0
- Peak End: 0.0
- Use normalization, if applicable is NOT checked

11.3.4.6.3 Peak Quality tab:

- Max Peak Height (MPH): 50,000RFU
- Homozygous Minimum Peak Height: 250.0
- Min. Peak Height Ratio: 0.6

11.3.5 Select the appropriate Panel:

11.3.5.1 GlobalFiler™: GlobalFiler_Panel_v1.1.1X

11.3.5.2 Yfiler™ Plus: Yfiler_Plus_Panel_v4.1X

11.3.5.3 GlobalFiler™ Express: GlobalFiler_Express_Panel_v1.4.1X

11.3.5.4 Yfiler™ Plus in Direct Mode: Yfiler_Plus_Panel_v4.1X

11.3.6 Select the Size Standard: GS600_LIZ_(60-460).

11.3.7 Analyze the samples. Save the project using, at a minimum, initials, case number and a descriptor such as run date, GF/YFP/GFE/YFPdm or number/other identifier (e.g. project # or injection #) in this format: “ABC 14-1234 Other Descriptor.”

11.3.8 Evaluate the data in accordance with CASE_5.

11.4 **Notes**

11.4.1 If a sample is ran multiple times (e.g. re-amplification, re-setup, etc.) and is present in the same project multiple times, the samples shall be clearly labeled during the appropriate step of the 3500 genetic analysis (reference DNA_8) so they can be distinguished from one another downstream in the GMID-X project (e.g. Item 1A_reamp, Item 1A_resetup, etc.).

11.4.2 Generally, each case will have its own separate GMID-X project. A “batch” project is allowed with prior technical manager approval; however, during review of cases in a batch project, the technical reviewer is responsible only for reviewing the samples in the case for which they are reviewing along with the case’s associated ladders and controls.

11.4.3 A quarterly check will be performed to ensure that the software settings remain aligned with policy specifications

Attachment(s):
None

Reference(s):
Life Technologies™ GeneMapper® *ID-X* Software Version 1.5 Reference Guide

Life Technologies™ GeneMapper® *ID-X* Software Version 1.6 User Bulletin

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

CASE_1 CASE NOTES (↑ Table of Contents)

Quick links:

1.1 Case Records

1.1.1 General case notes will be taken using applicable fields of the BEAST matrix panels, and a worksheet will be generated from the BEAST matrix panel information using the BEAST worksheet generator for incorporation into the final case record.

1.1.2 For DNA cases, analysts will use the Adobe PDF Portfolio program (portfolio) for case record organization. The portfolio is considered the "final case record" for all DNA cases and will be retained electronically in the BEAST.

1.1.2.1 All BEAST-generated case notes and DNA worksheets will be incorporated into the portfolio.

1.1.2.2 The DNA 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; Y-screen extraction worksheet, quantitation setup worksheet, and quantitation results (if applicable); downstream DNA extraction (Automate) worksheet; quantitation setup worksheet and quantitation results; normalization worksheet (if applicable); amplification setup worksheet(s); genetic analysis setup worksheet or 3500 Plate Grid Report; lot number worksheet; technical notes; electropherograms; results worksheet (DNA profile table); statistical calculations (if applicable); and any corrections performed as a result of review.

1.1.2.2.1 The **DNA_YS_Ext_Quant_Setup_Workbook_v1.0** may be used in lieu of the individual Y-screen extraction and quantitation worksheets, as applicable.

1.1.2.2.2 The **DNA_Quant_PCR_Setup_Workbook_v1.1** may be used in lieu of the individual quantitation and amplification worksheets, as applicable.

1.1.2.2.3 Additional folders within a portfolio are acceptable if case/analysis circumstances warrant, as necessary (e.g. STRbase microvariant confirmation).

1.1.3 For serology cases, if all information is contained within the BEAST matrix panels, the "final case record" can be either the BEAST-generated worksheet or a portfolio can be created (analyst discretion).

1.1.4 All original examination documentation (e.g. analysis worksheets, electropherograms, results, etc.) and the original (draft) report will be retained in the case record.

1.1.4.1 The retention of original examination documentation requiring amendment/correction will be accomplished by either 1) maintaining the original documentation in a "corrections folder" in the portfolio and/or 2) the correction made to the documentation will reflect the original as well as the change (i.e. strikethrough and initial method).

1.1.5 Examination documentation is considered complete when it is submitted for administrative and/or technical review, and any changes made to completed examination documentation during or after the review process shall be tracked in order to determine what was changed and who made the change.

1.1.5.1 Any required corrections discovered during administrative and/or technical review will be tracked by thorough documentation in the BEAST routing comments (history) detailing the corrections required for the case record identified during review (authored by the reviewer).

1.1.5.2 Any changes made to completed examination documentation as a result of the review process will be tracked by either 1) thorough documentation in the BEAST routing comments (history) detailing the changes made to the case record (authored by the case analyst, not the reviewer) and/or 2) using a “corrections folder” in the portfolio.

1.1.5.3 Any changes made to the report will be tracked by thorough documentation in the BEAST routing comments (history) detailing the changes made to the report.

1.1.5.4 When necessary, documentation in the BEAST routing comments (history) may also include making a note that directs an individual to the BEAST Narrative or Documents to view the required documentation (e.g. email).

1.2 **General Case Notes Requirements**

1.2.1 A description of the outer evidence packaging for each item of evidence.

1.2.2 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).

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

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

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

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

1.2.6 The use of any stereomicroscope and/or microscope search unless required by the test performed (e.g. Takayama, cell search).

1.2.7 The use of an alternative/ultraviolet light source.

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

1.2.9 If stains are produced from a liquid sample that has been submitted, the number and size or volume of stain(s) made.

1.2.10 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.

1.2.10.1 For all major crimes (person crimes) and for property crimes with a listed or known subject, prior written authorization allowing consumption of the questioned item(s) of evidence from the District Attorney's Office will be obtained and maintained in the case record.

1.2.10.2 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 questioned item of evidence for analysis.

1.2.10.3 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.

1.2.11 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 which controls and/or samples.

1.2.12 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.

1.2.13 If verification of analytical findings, such as sperm cell identification, are performed by another currently qualified analyst, they will be documented in the case notes and having the analyst initial/sign the appropriate page(s) in the case file.

1.3 **DNA-Specific Case Notes Requirements**

1.3.1 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.

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

- DNA Y-Screen Extraction Worksheet (**DNA_YS_Ext_Setup**)
- DNA Y-Screen Quantitation Worksheet (**DNA_YS_Quant_Setup**)
- DNA Extraction Worksheet (**DNA_AutoMate_Ext_Setup**)
- DNA Quantitation Worksheet (**DNA_Quant_Setup**)
- DNA Amplification Setup Worksheet (**DNA_GF_PCR_Setup**/**DNA_YFP_PCR_Setup**)
- Plate layout for amplifications in plates (**DNA_GF_YFP_Amp_Plate_Maps**)
- DNA 3500 Setup Worksheet (**DNA_3500_Setup**) or 3500 Plate Grid Report plate record with spectral listed
- DNA Lot Number Worksheet (**DNA_Lot_Numbers**)

1.3.2.1 The **DNA_YS_Ext_Quant_Setup_Workbook_v1.0** may be used in lieu of the individual Y-screen extraction and quantitation worksheets, as applicable.

1.3.2.2 The **DNA_Quant_PCR_Setup_Workbook_v1.1** may be used in lieu of the individual quantitation and amplification worksheets, as applicable.

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

1.3.3 GMID-X projects will contain all injections of samples and controls for each batch/case. The raw data is available in the raw data tab for each injection; therefore, it is retained with each sample/control in the GMID-X project.

1.3.4 Technical notes should be utilized to account for all injections as well as the evaluation and verification of all parameters, quantification standards, internal size standards, allelic ladders and analytical controls. Technical notes should list the reason(s) for any data that is reinjected and may be used to explain any deviations from protocol, other additional analysis, or to record reasons for decisions made about interpretation or terminating further analysis. Any injections that are not used for interpretation/reporting are to be accounted for in the technical notes. Alternatively, in lieu of technical notes, this documentation may be listed elsewhere in the case notes but must be present in the case record (portfolio).

1.3.5 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.

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

1.3.6.1 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 from and in what order it was generated (e.g. reinjection, re-setup, re-amplification, or re-extraction). 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 GMID-X project).

1.3.7 A results table must be included in the case record.

1.3.7.1 Analysts may report DNA profiles using either phenotypes or genotypes on a sample-by-sample basis; genotypes are most often reported in the OSBI forensic biology units.

1.3.7.2 Samples with results at a multi-copy locus (DYS385/DYF387S1) in Yfiler™ Plus will be reported using a comma.

1.3.7.3 Samples with no results at Y-Indel and DYS391 in GlobalFiler™ will be reported with either “NR” (no results) or “—“.

1.3.8 All statistical calculations performed must be included in the case record, if applicable.

1.3.9 At a minimum, all consumption photos, QuantStudio™ 5 quantitation data, 3500 Series Genetic Analyzer run folders, and GMID-X projects should be available for technical review and placed onto the secure, limited-access DNA server for storage.

1.3.9.1 The location will be listed by a general statement in the case record, such as “photo(s), QS5 data, 3500 data and GMID-X project(s) retained on the DNA server.”

1.3.10 CODIS Data Entry Forms must be included in the case record, if applicable, as verification of eligibility, correct DNA types, and correct specimen category for all profiles entered into CODIS.

1.3.10.1 Analysts shall follow the procedures outlined in CODIS_1 (Data Entry & Search Procedures) of the OSBI CODIS Unit Policy Manual for CODIS Data Entry Forms.

1.3.10.2 As a component of the verification for CODIS eligibility, information regarding the crime and the details of the item from which the profile was obtained will be documented in the case record. This may be accomplished by adding a narrative detailing consultation with the officer at the time of submittal or after submittal or by attaching an incident report or sexual assault history forms containing the pertinent information to the case record.

1.3.10.3 As a part of verifying CODIS eligibility, all samples for legal entry that are marked "No" for "DNA on file" on the OSBI Lab Submission Receipt (RFLE) should be queried using the CoDNA system, prior to forwarding to the CODIS Unit, to ensure they are not already in the database. The results of the CoDNA system search should be documented in the case record.

1.4 **Batching**

The OSBI forensic biology units may batch cases together. If cases are batched together, the following parameters should be maintained:

1.4.1 Personal identifying information from each case (e.g. suspect or victim names) should be maintained only in the appropriate case file.

1.4.2 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 case file.

1.4.2.1 Shared analysis worksheets with multiple case numbers present shall include the case number from the appropriate case file. It is not required to specifically indicate the case number (e.g. underline or circle since highlighting will not replicate when imaged), but this is recommended as it aids in the review process.

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

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

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

1.5 **Use of Technicians**

The OSBI forensic biology units may utilize qualified/authorized technicians in the laboratory for various laboratory functions, including assisting analysts with casework. If technicians are utilized in casework, or if another analyst is acting in the capacity of a technician, the analyst must clearly identify the technician and the steps performed by the technician in the case notes for each case by having the technician initial/sign the appropriate pages in the case file(s) either on the hard copy or electronically.

Attachment(s):

OSBI Forensic Biology Units' Policy Manual **CWQM_Abbreviation_List**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Quant_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YS_Ext_Quant_Setup_Workbook_v1.0**

OSBI Forensic Biology Units' Policy Manual **DNA_AutoMate_Ext_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_YFP_PCR_Setup**

OSBI Forensic Biology Units' Policy Manual **DNA_Quant_PCR_Setup_Workbook_v1.1**

OSBI Forensic Biology Units' Policy Manual **DNA_GF_YFP_Amp_Plate_Maps**

OSBI Forensic Biology Units' Policy Manual **DNA_3500_Setup** (optional)

OSBI Forensic Biology Units' Policy Manual **DNA_Lot_Numbers**

OSBI Forensic Biology Units' Policy Manual **CASE_OSBI_STATS_v2.2**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

CASE_2 DIGITAL PHOTOGRAPHY (↑ Table of Contents)

2.1 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.

2.2 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 and archived. For example, prior to consumption of a questioned item in testing or if the analyst feels that image(s) is/are essential for interpretation (e.g. blood spatter), the image should be archived (e.g. BEAST image vault, computer hard drive, DNA server) prior to proceeding to any subsequent analysis of the evidence.

2.3 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 renamed 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 “18-1234 1 Hair.”

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

2.5 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.

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

2.7 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.

2.8 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.

2.9 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.

2.10 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.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI Forensic Biology Units’ Training Manual

CASE_3 SEROLOGY ANALYSIS (↑ Table of Contents)

Quick links:

3.1 Blood Analysis

3.1.1 Analysts may use sample selection for testing of bloodstains 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.

3.1.2 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.

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

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

3.2 Sexual Assault Analysis

3.2.1 For sexual assault kits and evidence collected from the Office of the Chief Medical Examiner, cuttings from up to two intimate swabs (body swabs) of the same type (origin) should be combined into a single tube for the purposes of serological testing (p30 and sperm cell search); this includes swabs from both orifice and non-orifice body areas, respectively. For example, cuttings from two vaginal swabs (orifice) may be combined into a single tube for testing, or cuttings from two thigh swabs (non-orifice) may be combined into a single tube for testing, etc. Swabs that are not clearly indicated as from the same area/staining (or if that determination cannot be made) will not be combined for analysis.

3.2.2 Liquid samples (e.g. urine, aspirate, etc.) will be homogenized for sexual assault analysis and sampled as follows:

3.2.2.1 Determine the portion (volume) of the received sample to be used for testing. Vortex (homogenize) the sample, then remove the volume determined to be tested and transfer it to another tube.

3.2.2.2 Vortex the portion transferred to the new tube for testing and then spin down to pellet any potential cellular material present.

3.2.2.3 Test the sample using the procedures outlined in SER_5 and SER_6, as applicable.

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

3.2.4 Sample selection 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 the stains selected for testing are all negative.

3.2.4.1 Additional stains may need to be added and tested until a positive p30 or cell search result is obtained or all stains have been subjected to confirmatory testing.

3.2.4.2 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.

3.2.4.3 If terminating testing of an item after AP Spot Test negative results without using sample selection to test stains using p30/cell search testing, approval should be obtained from the technical manager or Lead Analyst and available in the case record.

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

3.2.6 Regardless of p30 testing results, cell search must be conducted for items not tested with the Y-screen assay.

3.2.7 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. If an item is tested with the Y-screen assay, any smear slides present with the case do not need to be analyzed, regardless of the Y-screen results (unless they are inconclusive), but should be retained with the evidence.

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

3.2.9 Serological sexual assault analyses, such as p30 and cell search testing, are not required for cases/items processed with the Y-screen assay.

3.2.9.1 P30 and/or cell search will only be performed on a case-by-case basis for cases that have already been subjected to the Y-screen assay, and only upon written request by the District Attorney's Office, which shall be maintained in the case record.

3.3 **Hair Analysis**

3.3.1 Due to the nature of hair transfer, the OSBI forensic biology units will not perform hair examinations in cases where individuals have routinely or recently had close contact or access to common areas (e.g. living quarters, vehicles, etc.).

3.3.2 If other probative evidence exists in a case, no hair examination will be performed unless case circumstances dictate otherwise. For example, if semen is identified in a rape kit, any hairs present in the rape kit will typically not be examined.

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

Attachment(s):
None

Reference(s):
SWGDAM Guidelines for the Collection and Serological Examination of Biological Evidence

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

OSBI Forensic Biology Units' Training Manual

CASE_4 DNA ANALYSIS (↑ Table of Contents)

Quick links:

4.1 Y-Screen Assay Considerations

4.1.1 The Y-screen assay, a "Direct to DNA" approach, may only be used for intimate forensic orifice swab samples (questioned items only) that are included in standard sexual assault kits, packages designated as sexual assault kits, or collected from the Office of the Chief Medical Examiner in instances where a sexual assault is suspected. Examples of intimate forensic orifice swab samples include but are not limited to vaginal swabs, external genitalia swabs (encompassing mons pubis, labia majora, labia minora, urethra, hymen, posterior fourchette, perineal, perineum, prepuce, clitoral hood, clitoris, etc.), anal swabs, oral swabs, etc.

4.1.2 The Y-screen assay will not be used for:

4.1.2.1 Any samples that do not meet the requirements listed above, including but not limited to, intimate external (non-orifice) body swabs (not including external genitalia swabs), etc. These sample types are more vulnerable to sampling variability and may produce false-negative Y-screen assay results.

4.1.2.2 Any samples that do not meet the requirements listed above, including but not limited to, underwear, clothing, bedding, tampons, maxi pads, etc. (including swabs of such substrates). These sample types have shown variability in substrate composition and fabric dyes, where applicable, that may affect (inhibit) amplification by real-time PCR. Because the Y-screen assay makes use of a crude lysis, PCR inhibitors cannot be removed during the assay. These sample types are also vulnerable to sampling variability and may produce false-negative Y-screen assay results.

4.1.2.3 Any sample requiring consumption. Instead, the sample must be analyzed using the appropriate serological procedures (p30 and cell search) to ensure a sperm slide is prepared prior to consumption.

4.1.2.4 Cases involving genetically male victims and/or genetically female suspects as the procedure is not applicable.

4.1.3 Cuttings from up to two orifice swabs of the same type (origin), i.e. intimate swabs collected from the same orifice of an individual's body, such as vaginal swabs, external genitalia swabs, anal swabs, etc., should be combined into a single tube for the purposes of screening for human male DNA using the Y-screen assay. Swabs that are not clearly indicated as from the same orifice/staining (or if that determination cannot be made) will not be combined for analysis.

4.1.4 Samples subjected to the Y-screen assay should be identified during analysis by including them on designated Y-screen assay-related worksheets (or in an appropriate Excel workbook attached to the policy manual). The identifier "YS" may also be used in the case notes (e.g. "Item # YS"). Dashes and/or underscores may be added as necessary or at analyst preference.

4.1.5 Y-screen samples positive for the presence of male DNA are candidates for additional "downstream" DNA testing. For any candidate sample(s) selected for STR and/or Y-STR analysis (analyst discretion), the type of DNA extraction that will be performed shall be determined based on case information available to the analyst at the time of analysis relevant to each sample under consideration, including but not limited to, case scenario, sexual assault history information, victim statements, officer reports, etc.

4.1.5.1 If case information indicates seminal fluid may be present, a differential extraction will be performed using a new cutting/cutting(s) from the original evidence item(s).

4.1.5.1.1 In cases where there is supporting information that a penis was exposed during an alleged assault, regardless of whether ejaculation is indicated as having occurring or not, the penile exposure in itself indicates that seminal fluid may be present.

4.1.5.2 If case information indicates seminal fluid is not suspected to be present, a standard (non-differential) extraction will be performed using a new cutting/cutting(s) from the original evidence item.

4.1.5.3 If the case information is unclear as to whether seminal fluid may be present on an item or not, the type of extraction to be performed will be handled in consultation with the technical manager and/or Lead Analyst using a new cutting/cutting(s) from the original evidence item(s).

4.1.5.4 Clear documentation must be included in the case record to support the decision of the analyst regarding the type of DNA extraction to use.

4.1.6 If male DNA is not detected with the Y-screen assay, the IPC amplification plot must be assessed to confirm the reaction did not fail due to the presence of inhibitors.

4.1.7 Samples with inconclusive results during the Y-screen assay should proceed for downstream DNA analysis to remove inhibitors.

4.1.8 No further analysis (serology or DNA) is necessary for negative Y-screen samples.

4.1.9 The Y-screening assay is only intended to be used as a screening tool for the detection of human male DNA. Due to the crude lysis NaOH extraction used, any resulting quantification data should not be used for additional downstream decisions regarding STR and/or Y-STR analysis. Only after additional extraction and quantification using downstream DNA analysis

methods will quantitation values, M:F ratios, and degradation index information be interpreted to assess sample viability for STR and/or Y-STR analysis.

4.2 Extraction Considerations

4.2.1 The table below lists guidelines for the amount of sample to use for DNA extraction (per tube), storage/preservation, special handling/preparation procedures, and extraction kit. However, as each sample will be different, it is analyst discretion to use an appropriate amount of sample for DNA 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 the action.

Sample Type	Sample Requirement	Storage/ Preservation	DNA Origin	Special Handling Procedures/ Preparation	PrepFiler™ Kit
Liquid Samples (Whole Blood, Saliva)	Up to 40µL	Prepare stain & 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 ½ 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 & rinse with deionized water. Freeze, then cut or break to expose the inner surface & remove soft tissue & 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 & 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 & rinse with deionized water prior to extraction. If mounted, use xylene to remove mounting media.	Express
Miscellaneous Swabs	~½ 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

***** Samples testing presumptive or confirmatory positive for seminal fluid.

4.2.2 Swabs of items from the same area/staining (e.g. vaginal swabs, bloodstain from doorjamb, etc.) may be combined for the purposes of DNA analysis regardless of serology results. For example, two vaginal swabs with differing results (swab 1A1 – p30 positive/sperm negative and swab 1A2 – p30 positive/sperm positive) may be combined for DNA analysis, if necessary. However, 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.

4.2.3 Samples specifically designated for the purpose of serving as secondary known reference samples for an individual including, but not limited to, cigarette butts, toothbrushes, hairs, gum, etc., may be processed as known samples throughout the DNA analysis process.

4.3 Amplification Considerations

4.3.1 In some cases of differential extractions of intimate swabs, it may not be necessary to amplify the epithelial fraction in GlobalFiler™ so long as the analyst can articulate the only information likely to be obtained from the analysis of the epithelial fraction with GlobalFiler™ would be to establish the swab was collected from the indicated individual based on the quantitation results. The analysis performed on the sample must be adequately documented (e.g. in the technical notes, with the quantitation results, or on the amplification worksheet) and reported to the customer.

4.4 DNA Profile Considerations

4.4.1 In the event that there is evidence of peaks below analytical threshold and above the limit of detection, 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 amplification at a higher target 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 would cause other interpretation problems that may result from off-scale data, then no further analysis may be necessary.

4.4.2 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 reinject, re-setup, or re-amplify a sample if not all the peaks are

above analytical threshold. However, results inconsistent with a background profile must be handled according to CASE_4.4.1 above.

4.4.3 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 size standard (ILS) peaks reveals the sample injected properly, the sample does not need to be reinjected. Re-amplifying the sample may be necessary if a quantitation value was obtained which may happen with an extremely degraded sample.

4.4.4 If non-concordant results occur, the profile should be reinjected 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 reinjecting or re-amplifying the sample.

4.4.5 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.

4.5 Monitoring Analytical DNA Procedures Using Controls & Standards (Frequency & Tolerance of Controls)

4.5.1 Reagent Blank Control Requirements

4.5.1.1 Analytical procedures will be monitored using reagent blank controls ("reagent blanks") analyzed in the same manner as the associated forensic samples.

4.5.1.2 Reagent blank naming will include an indicator that associates the reagent blank with either Y-screen samples or questioned or known samples extracted for STRs and/or Y-STRs. Acceptable naming conventions include the following (appropriate number, dashes and/or underscores may be added, as necessary, or at analysts' preference):

4.5.1.2.1 **RBYS1** or **RB1YS**: Reagent blank #1 for Y-screen assay of questioned sample(s)

4.5.1.2.2 **RBQ1**: Reagent blank #1 for standard extraction of questioned sample(s)

4.5.1.2.3 **RBQE1/RBQS1** or **RBE1/RBS1** or **RBQ1E/RBQ1S**: Respective fractions of reagent blank #1 for differential extraction of questioned sample(s)

4.5.1.2.4 **RBK1**: Reagent blank #1 for standard extraction or direct amplification of known sample(s)

4.5.1.3 Analysts should thoroughly evaluate their extraction batch(es) to determine the appropriate number of reagent blanks needed for each extraction set based on their training and experience. At least one reagent blank must be included with every set of extractions, and all reagent blanks should be placed at the end of the extraction set.

4.5.1.4 If extraction sets are split into mini-sets, the reagent blank for each mini-set should be placed after the respective mini-set and remain designated to those samples throughout processing. Example: An extraction with four cases may be split into two mini-sets – one with low amounts of DNA expected (e.g. touch DNA samples) and one with high amounts of DNA expected (e.g. bloodstains). The reagent blanks will be placed immediately after each mini-set and will be associated with the mini-set throughout the DNA analysis process:

Item 1	Item 2	Item 3	RBQ1	RBQ2	Item 4	Item 5	Item 6	RBQ3	RBQ4

In this example, RBQ1 and RBQ2 are associated with items 1-3 and RBQ3 and RBQ4 are associated with items 4-6.

4.5.1.5 Reagent blank controls associated with each extraction or direct amplification set shall be extracted/amplified concurrently (at the same time) and treated in the most sensitive conditions (volume) as the forensic sample(s) of the set.

4.5.1.6 Sometimes it may be necessary to split the extraction of a sample across multiple extraction tubes. Example: If four vaginal swabs have very little sperm detected and/or very faint p30 results, it may be desirable to extract all four swabs, but this would exceed the space available in any single extraction tube, requiring the swabs to be extracted among different tubes and then their resulting extracts combined. In such instances, one reagent blank is acceptable for this set of simultaneous extractions. However, if an analyst decides to combine two extracts from two separate extraction sets (different times/dates), then the reagent blanks from each extraction set must be combined as well.

4.5.1.7 With the exception of Y-screen reagent blanks and direct amplification reagent blanks, all reagent blanks should be brought to a working volume of ~50µL, if necessary. Sometimes reagent blanks may be brought to a working volume of ~15-20µL depending on their related sample types and based on analyst discretion.

4.5.1.8 If samples are manipulated after extraction, at least one reagent blank must undergo the same manipulation. For example, if a sample is reconstituted or concentrated, at least one of the reagent blanks associated with that extraction set must also follow through that process. Alternatively, with technical manager and/or Lead Analyst consultation and approval, an additional reagent blank(s) may be introduced to control for any subsequent manipulation of sample(s) within the extraction batch as long as an original reagent blank associated with the extraction set and an additional reagent blank are both amplified and typed in accordance with this policy.

4.5.1.9 With the exception of direct amplification reagent blanks, all reagent blanks must be quantitated.

4.5.1.9.1 If all evidentiary sample processing for a given extraction set will be terminated at the quantification stage for any reason, in order to monitor analytical quality, the reagent blank(s) must be either quantified or typed in order for the evidentiary sample processing to be terminated.

4.5.1.10 Y-screen reagent blanks will not be amplified or typed.

4.5.1.11 For differential extractions that result in a reagent blank(s) for each fraction, the reagent blank(s) from each fraction shall be independently evaluated with the corresponding fraction.

4.5.1.12 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.

4.5.1.13 If a reagent blank in an extraction set demonstrates any signal during quantitation, the reagent blank with the greatest signal must be amplified and characterized. *General guidance* is provided below; however, if results outside of

these parameters occur, or an analyst is unsure how to proceed, consultation with the technical manager is required and should be documented prior to proceeding with additional analysis:

4.5.1.13.1 If a reagent blank produces signal in any target (LA, SA and/or Y), the reagent blank with the greatest signal should be amplified.

4.5.1.13.2 If amplifying using GlobalFiler™, the reagent blank with the greatest signal in LA, SA and/or Y target should be used.

4.5.1.13.3 If amplifying using Yfiler™ Plus, the reagent blank with the greatest signal in Y target should be used.

4.5.1.13.4 If amplifying in both GlobalFiler™ and Yfiler™ Plus, the reagent blank with the greatest signal should generally be amplified using GlobalFiler™. However, based on the needs of the extraction set, including treatment of the reagent blank for amplification in the most sensitive conditions as the forensic samples(s) of the extraction set (i.e. concentration), there may be times when the reagent blank should be amplified using Yfiler™ Plus instead.

4.5.1.14 If a sample is concentrated, at least one reagent blank associated with the sample must also be concentrated. The final concentration volume of a reagent blank (or sample) used for amplification may not be less than the maximum volume of the amplification kit to be used.

4.5.1.15 When amplifying extraction sets, an associated reagent blank(s) must be amplified using the same typing test kit, instrument model, and sensitivity conditions as required by the forensic sample(s) within the extraction set containing the least amount of DNA.

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

4.5.1.17 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 volume amplified 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. However, if a sample is re-amplified with the same amplification kit but the template volume increases over that of the original reagent blank, the reagent blank will be re-amplified with the increased volume.

4.5.1.18 Reagent blanks associated with each extraction set must be typed utilizing the same instrument model and the most sensitive injection conditions of the extraction set.

4.5.1.18.1 Reagent blanks must be injected at 15 seconds for GlobalFiler™, Yfiler™ Plus, GlobalFiler™ Express, and Yfiler™ Plus Direct Mode.

4.5.1.18.2 If any samples are injected at an increased injection time (i.e. 24 seconds), the associated reagent blank(s) must also be injected at the increased injection time.

4.5.1.19 With multiple amplification test kits available for use, if all reagent blank(s) associated with an extraction set or sample being amplified have been depleted,

there shall be no additional analysis using a different amplification test kit without an associated reagent blank available.

4.5.1.20 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.

4.5.2 **Quantitation & Amplification Control Requirements**
Analytical procedures will be monitored using the following analytical controls, which will be analyzed in the same manner as their associated forensic samples:

4.5.2.1 **Quantitation Negative Control (QNC)**

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

4.5.2.1.2 The QNC should be "undet." during quantitation (no signal).

4.5.2.1.3 If the QNC displays a signal, it will be marked as "Unknown" and reanalyzed to determine the amount of DNA detected. The technical manager and/or Lead Analyst shall be notified and determine if the plate is acceptable or if it will be re-setup and rerun.

4.5.2.2 **Amplification Negative Control (NC)**

4.5.2.2.1 This control must be included with every amplification set and shall be amplified concurrently using the same typing test kit on the same instrument as the samples.

4.5.2.2.2 Each negative amplification control shall be typed.

4.5.2.2.3 The negative amplification control used by the OSBI forensic biology units will substitute TE^{-4} in place of sample.

4.5.2.2.4 The negative amplification control should have no observable peaks above the analytical threshold. If peaks are detected, then reference 4.5 below.

4.5.2.3 **Amplification Positive Control (PC)**

4.5.2.3.1 This control must be included with every amplification set and shall be amplified concurrently using the same typing test kit on the same instrument as the samples.

4.5.2.3.2 Each positive amplification control shall be typed.

4.5.2.3.3 The OSBI forensic biology units will use the control DNA provided in the GlobalFiler™, Yfiler™ Plus, and GlobalFiler™ Express PCR Amplification Kits (DNA Control 007).

4.5.2.3.4 The positive amplification control should yield the expected male DNA profile for DNA Control 007.

4.5.2.3.5 If the positive control does not yield the correct male DNA profile, the sample should be re-setup and the technical manager and/or Lead Analyst will be notified.

4.5.3 Allelic Ladder & Internal Size Standard Requirements

4.5.3.1 Allelic ladders and internal size standards must be used to appropriately assign DNA types to fragments produced in PCR-based systems.

4.5.3.2 Allelic ladders and internal size standards should not contain any unexplained peaks that exceed the analytical threshold within the analysis range.

4.6 Contamination/Unexplained Peaks

4.6.1 General Considerations

4.6.1.1 The OSBI forensic biology units shall monitor for contamination, follow decontamination procedures to minimize/prevent contamination, and follow procedures for interpreting data potentially affected by contamination.

4.6.1.2 The OSBI forensic biology units shall follow procedures for uniformly evaluating controls and samples to verify expected results as well as remediating instances of control failure or sample contamination.

4.6.1.3 Interpretation of data potentially affected by contamination or failed PCR controls will be evaluated on a case-by-case basis in conjunction with the technical manager and unit supervisor. The data provided by the OSBI internal validation of interpretation thresholds will be taken into account during these types of evaluations.

4.6.2 Monitoring for Contamination

4.6.2.1 Signal observed in Y-screen reagent blanks during quantitation may not be considered contamination unless observed in the Y target; however, the technical manager shall be notified if any target(s) have signal during quantification (i.e. small autosomal, large autosomal, and/or Y).

4.6.2.2 If signal is detected in the Y target for a Y-screen reagent blank, the data from the quantification plate will not be used. All samples associated with the affected reagent blank may be re-quantitated, re-analyzed (i.e. re-extracted and re-processed through a new Y-screen assay), processed using traditional serology methods, or forwarded on for downstream DNA testing, depending on the needs of the case. Analysts shall consult with the technical manager to determine how to proceed when a Y-screen reagent blank has signal in the Y target.

4.6.2.3 All amplified samples shall be evaluated for potential contamination. Although not always readily apparent, unlike in controls, contamination shall be monitored through sample data analysis. For example, a forensic questioned sample with a very low quantitation value or an undetected (zero) quantitation value yielding an off-scale profile could be an indication of a sample switch. Another example includes a known reference sample yielding a mixture, which could be an indication of possible contamination.

4.6.2.4 With the exception of Y-screen reagent blanks, signal observed in reagent blanks during quantitation may not be considered contamination unless any called peaks above analytical threshold are obtained.

4.6.2.5 Possible peaks below analytical threshold may not be considered contamination in the absence of any called peaks.

4.6.2.6 Any time an evaluation of controls yields unexpected results, the control shall be re-injected to verify the result. If the reinjection is acceptable, then the control shall be considered valid. If the reinjection is not acceptable, the incident will be brought to the attention of the technical manager and unit supervisor and handled in accordance with OSBI CSD QP 13 (Nonconforming Work) and the procedures outlined in this policy manual.

4.6.3 Detection & Control of Contamination

4.6.3.1 Analysts will adhere to the procedures within this policy manual, including QC_1 (Cleaning, Decontamination & Sterilization), in an effort to limit the potential for contamination. When necessary, following a contamination event, the affected unit(s) shall follow a decontamination procedure determined by the technical manager that is proportional to the level/type of contamination observed.

4.6.3.2 If possible contamination is observed, the technical manager and unit 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 (Nonconforming 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.

4.6.3.3 Based on the OSBI forensic biology units' internal validation, no peaks below the following limit of detection thresholds need to be evaluated as being attributable to contamination:

Limit of Detection (RFU)	GlobalFiler™ 7s/15s	GlobalFiler™ 24s	Yfiler™ Plus 7s/15s	Yfiler™ Plus 24s
Blue	50	50	50	75
Green	50	50	50	75
Yellow	50	50	50	75
Red	50	50	50	75
Purple	50	50	50	75

Limit of Detection (RFU)	GlobalFiler™ Express 7s/15s	GlobalFiler™ Express 24s	Yfiler™ Plus Direct Mode 7s/15s	Yfiler™ Plus Direct Mode 24s
Blue	60	60	60	60
Green	60	60	60	60
Yellow	60	60	60	60
Red	60	60	60	60
Purple	60	60	60	60

4.6.3.4 Reagent blanks and negative controls with only one peak above the limit of detection but below the analytical threshold may be used for reporting. The peak must be documented in the technical notes and the technical reviewer must agree.

4.6.3.5 Reagent blanks and negative controls with two or more peaks above the limit of detection but below the analytical threshold or with any called peaks not attributable to an artifact will be evaluated on a case-by-case basis in conjunction with the unit supervisor and the technical manager. During this evaluation, the technical manager, or designee, may review the spreadsheet of DNA profiles from employees.[26]

4.6.3.6 If a contaminant profile is observed and not attributable 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.

4.7 Criminal Paternity Cases

4.7.1 Paternity cases should be processed analyzing the alleged father, alleged mother and offspring. Paternity case requests without the full trio of individuals will be accepted and worked on a case-by-case basis in consultation with the technical manager and/or Lead Analyst.

4.7.2 Paternity case samples (known buccal swabs) will be processed as known samples throughout the DNA analysis process, as they are reference samples from individuals in the case and are expected to contain a high amount of DNA.

4.7.3 Paternity cases with known reference samples other than buccal swabs (e.g. tissue products of conception) will be considered for acceptance and analysis only after consultation with and approval from the unit supervisor and/or technical manager and/or Lead Analyst.

4.8 Forensic Genetic Genealogy (FGG)

4.8.1 Forensic genetic genealogical DNA analysis and searching (FGGS) differs from traditional forensic STR DNA analysis in both the type of technology employed (single nucleotide polymorphisms, 'SNPs') and the nature of the databases utilized (publicly-available open-data personal genomics database or a direct-to-consumer genetic genealogy service, i.e. third-party SNP databases).

4.8.1.1 FGGS is not considered subcontracting since the OSBI does not perform this service and does not evaluate the resulting data for any purpose (e.g. determination for NDIS eligibility).

4.8.1.2 FGGS is not forensic familial DNA searching, as familial searching is an additional search of a law enforcement DNA database (e.g. CODIS), which is conducted after a routine search of the crime scene evidence DNA profile has been completed with no resulting matches. FGGS begins with a search of a third-party SNP database and uses a different search algorithm.

4.8.2 At any time, the OSBI may independently select suitable forensic samples from eligible cases to send to a private vendor laboratory for the purposes of FGGS.

[26] The OSBI Forensic Biology Discipline Employee DNA Profile Spreadsheet is a limited access, controlled Excel spreadsheet, uniquely identified and verified for accuracy, located on the secure Biology server. It consists of DNA profiles from OSBI employees and family members that have provided a buccal swab for inclusion in this reference spreadsheet for comparison purposes (i.e. contamination, training, etc.), when necessary.

4.8.3 Any outside investigative agency may formally request (in writing) assistance from the OSBI in determining whether their case has the potential for FGGS.

4.8.4 Potential cases/samples selected for FGGS will be evaluated according to this policy and in accordance with the criteria provided in the U.S. Department of Justice Interim Policy on Forensic Genetic Genealogical DNA Analysis and Searching and the recommendations in the SWGDAM Overview of Investigative Genetic Genealogy.

4.8.5 **Case Criteria for FGGS**
Case requirements when determining FGGS suitability shall include:

4.8.5.1 Cases involving an unsolved violent crime (homicide or sexual assault) with a candidate forensic sample from a putative perpetrator, or cases involving the unidentified remains of a suspected homicide victim (i.e. unidentified human remains);

4.8.5.1.1 A district attorney may authorize the investigative use of FGGS for violent crimes or attempts to commit violent crimes other than homicide or sexual offenses when the circumstances surrounding the criminal act(s) present a substantial and ongoing threat to public safety or national security.

4.8.5.2 Candidate forensic samples must be from a single source or deduced single source and attributable to the person of interest;

4.8.5.3 Prior to performing FGGS, the forensic DNA profile from the candidate forensic sample (i.e. crime scene evidence DNA profile) must have been uploaded to CODIS, and subsequent CODIS searches must have failed to produce a probative and confirmed DNA match in SDIS or NDIS;

4.8.5.4 The investigative agency with jurisdiction of either the crime or the location where the unidentified human remains were discovered (if different) must have pursued reasonable investigative leads (as determined by the technical manager, Lead Analyst, or designee) to solve the case or to identify the unidentified human remains; and

4.8.5.5 Where applicable, relevant case information must have been entered into the National Missing and Unidentified Persons System (‘NamUs’) and the Violent Criminal Apprehension Program (‘ViCAP’) national database.

4.8.6 **Evaluating Sample Suitability for FGGS**

4.8.6.1 If each of the case criteria set forth above have been satisfied, and the forensic DNA profile was uploaded to CODIS by the OSBI, the technical manager, Lead Analyst, or designee, shall perform the following evaluations and document them in the case record:

4.8.6.1.1 Determine if the candidate forensic sample is from a single source donor or is a deduced mixture;

4.8.6.1.2 Assess the candidate forensic sample’s suitability (e.g. quantity, quality, degradation, mixture status, etc.), considering the private vendor laboratory’s requirements and/or recommendations for performing FGGS analysis, and advise the investigative agency about the results of this evaluation; then

4.8.6.1.3 If applicable, advise the investigative agency of any reasonable scientific alternatives to FGGS given the nature and condition of the candidate forensic sample, and the availability of other DNA technologies or techniques.

4.8.6.2 If each of the case criteria set forth above have been satisfied, and the forensic DNA profile was uploaded to CODIS by another NDIS laboratory other than the OSBI, the technical manager, Lead Analyst, or a designee, will contact the designated official from that laboratory for assistance with the aforementioned evaluations. This consultation shall be documented in the case record.

4.8.7 If a case is suitable for FGGS and contains an associated sample adequate for analysis, prior to proceeding, applicable legal representatives, such as the district attorney (or OSBI legal counsel) shall be contacted by the investigating agency and advised of the nature and status of the investigation, the results of the laboratory's evaluation of the candidate forensic sample, and any reasonable scientific alternatives to FGGS. The investigating agency may request that OSBI act on their behalf; this should be documented in the case record, if available. The district attorney and the investigative agency (or designee) must agree that the candidate forensic sample is suitable for FGGS, and that it is a necessary and appropriate step at that stage of the investigation to develop investigative leads or identify the unidentified human remains. If agreement is reached on these points, approval should be documented in the case record, if available, and FGGS may proceed.

4.8.8 In accordance with the OSBI CSD Quality Manual, when evidence is sent to a private vendor laboratory for FGGS, the OSBI shall notify the requesting agency in writing (e.g. report, letter or memorandum) and the chain-of-custody shall be tracked.

4.8.9 In the event the OSBI enters and searches a FGG profile in a third-party database, the OSBI shall identify itself as law enforcement and enter and search FGG profiles only in those databases that provide explicit notice to their service users and the public that law enforcement may use their service sites (i.e. online web page and content of a database service) to investigate crimes or to identify unidentified human remains. The OSBI shall, if possible, configure service site user settings that control access to the FGG profile data and associated account information in a manner that will prevent it from being viewed by other service users.

4.8.10 Information derived from FGGS genetic associations may be used by law enforcement only as an investigative lead.

4.8.11 If there is a genetic association of a FGG profile with a service user, including the identification through subsequent genealogy research of one or more third parties who may have a closer kinship relationship to the donor of the forensic sample than the associated service user, known reference samples should be obtained from these individuals, if possible, for the purpose of conducting FGGS to help identify the donor of the forensic sample.

4.8.12 Once a person of interest is identified following the use of FGGS, a known reference sample should be obtained and submitted for STR DNA analysis. The suspect's STR DNA profile must be directly compared to the forensic profile previously uploaded to CODIS to confirm that the forensic sample could have originated from the suspect.

4.8.13 **Sample & Data Control and Disposition**

4.8.13.1 All FGG profiles and genetic genealogy service account information and data shall be treated as confidential in accordance with any applicable laws, regulations, policies, and procedures.

4.8.13.2 If a suspect is arrested and charged with a criminal offense while FGGS is in progress, the relevant vendor laboratory or direct-to-consumer database service should be contacted by the investigating agency or their designee and directed to cease all testing at a point in time when the (forensic or reference) sample (and any derivative DNA products) can be preserved and returned.

4.8.13.3 Once a person of interest is included by STR DNA analysis, the crime scene or unidentified human remains sample should be expunged from the third-party SNP database(s), including the FGG profile and all associated account information and data by the investigating agency or their designee.[27] This may be performed after completion of criminal proceedings, if applicable.

4.8.13.4 Once a person of interest is included by STR DNA analysis, surreptitious or abandoned collections of targeted reference samples should be expunged from the third-party SNP database(s), including the FGG profile and all associated account information and data by the investigating agency or their designee.

4.8.13.5 Subsequent to applicable law, in all cases that result in a criminal prosecution, reference samples obtained from third parties for FGGS (including all derivative DNA products), all derivative FGG profiles, and all genetic genealogy service account information and data shall be destroyed, only after the entry of an appropriate judicial order, under the direction of the investigating agency or their designee. The authorized destruction of these samples, profiles, information, and data shall be documented.

4.8.13.6 Subject to applicable government information retention schedules, if FGGS does not result in an arrest and the filing of criminal charges, all third-party reference samples (including all derivative DNA products), all derivative FGG profiles, and all genetic genealogy service account information and data shall be destroyed after their investigative use is complete under the direction of the investigating agency or their designee. The destruction of these samples, profiles, information, and data shall be documented.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

United States Department of Justice Interim Policy Forensic Genetic Genealogical DNA Analysis and Searching

SWGDAM Overview of Investigative Genetic Genealogy

SWGDAM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits

OSBI Forensic Biology Units' Training Manual

[27] Note, all FGG profiles, account information, and data shall be retained by the investigative agency for potential use during prosecution and subsequent judicial proceedings.

CASE_5 DNA INTERPRETATION & COMPARISON GUIDELINES[28] (↑ Table of Contents)

Quick links:

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[28] Interpretation of results in casework is a matter of professional judgement 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. The following 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. Tools used for interpretation are addressed in this section of the policy manual as listed in the "Quick links" above (**bolded**).

5.1 DNA typing results should be “verified and interpreted” (SWGDAM Interpretation Guidelines) by reviewing peak designations and other software-generated information, internal size standards, ladders, positive amplification controls, negative amplification controls, reagent blanks, and samples.

5.2 **Evaluation of Associated Standards, Ladders & Controls**
Analysts should review and verify the following:

5.2.1 **Software-Generated Information**

5.2.1.1 Correct spectral

5.2.1.2 Correct panel

5.2.1.3 Correct size standard

5.2.1.4 Correct genotyping parameters (Analysis Method)

5.2.2 **Internal Size Standard & Allelic Ladders**

5.2.2.1 The internal size standard performed as expected and is present in all ladders, controls, and samples.

5.2.2.2 All ladders selected for comparison to controls and samples performed as expected with all alleles sized and labeled correctly.

5.2.3 **Extraneous/Unexplained Peaks**

5.2.3.1 All profiles will be evaluated to ensure no extraneous or unexplained peaks are present within the analysis range of 60-460bp for GlobalFiler™ and Yfiler™ Plus.

5.2.3.2 Samples containing unexplained peaks will be handled in accordance with the applicable policies of this manual.

5.2.4 **Analytical Controls: Positive/Negative Amplification Controls & Reagent Blanks**

5.2.4.1 The positive and negative amplification controls and all reagent blanks will be verified to ensure that the results obtained are acceptable.

5.2.4.2 The correct profile must be obtained for the positive amplification control.

5.2.4.3 The negative amplification control and reagent blank(s) must have no peaks indicating the presence of contamination per CWQM_9 (Analytical Procedures) and CASE_4 (DNA Analysis).

5.3 **Interpretation of Non-Allelic Peaks/Possible Artifacts**

The following are defined as artifacts (non-allelic peaks) by the OSBI forensic biology units and will be designated as indicated. 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 and/or Lead Analyst.

5.3.1 **PCR Products**

5.3.1.1 **Stutter**

5.3.1.1.1 Generally, when the height of a peak in the stutter position exceeds the GMID-X stutter expectations for a given locus, the peak is consistent with being of allelic origin and should be designated as an allele.

5.3.1.1.2 The marker-specific stutter ratios in the GMID-X software stutter panels shall be utilized based on the stutter ratios determined during the OSBI forensic biology units' supplemental stutter filter studies. If marker-specific stutter ratios are unavailable (e.g. n ± 2), analysts shall rely on training, experience, and validation data for the determination of whether a peak is categorized as stutter.

5.3.1.1.3 With higher than recommended amounts of template DNA, stutter may sometimes be called as a peak by the 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.

5.3.1.1.4 In the OSBI forensic biology units' internal validation, it was observed that the stutter percentage generally increases with allele length.

5.3.1.1.5 Combined stutter (n + X and n – X) may be present in samples. If the calculated combined stutter percentage is at or below the additive value of the plus stutter and the minus stutter, the peak may be re-labeled as "additive stutter."

5.3.1.1.6 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.

5.3.1.1.6.1 The presence of n + 3 / n + 4 / n + 5 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 the OSBI forensic biology units' internal validation of interpretation thresholds.

5.3.1.1.7 Analysts will rename confirmed stutter peaks as “ST” or “AST,” as applicable, in the GMID-X project. Analysts may document the stutter peak ratio(s) and the marker-specific ratio(s).

5.3.1.1.8 The technical reviewer must agree with all stutter determinations.

5.3.1.2 **Minus A (Non-Template Nucleotide Addition)**

5.3.1.2.1 With higher than recommended amount of template DNA, lack of non-template nucleotide addition (i.e. minus A [n – 1] peaks) may be observed as a “shoulder” of the main allele peak.

5.3.1.2.2 In the OSBI forensic biology units’ internal validation, minus A was observed in Yfiler™ Plus amplifications when greater than 0.5ng was amplified.

5.3.1.2.3 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.

5.3.1.2.4 Alternatively, if interpretations are not affected by the minus A, analysts should rename minus A as “-A” in the GMID-X project.

5.3.1.2.5 If samples with minus A are interpreted, analysts shall not utilize any quantitative aspects of interpretation (such as stutter and peak height ratios).

5.3.1.2.6 The technical reviewer must agree with all minus A determinations.

5.3.1.3 **Trailing Primer Peaks**

5.3.1.3.1 Analysts should rename trailing primer peaks as “TP” in the GMID-X project.

5.3.1.3.2 The technical reviewer must agree with all trailing primer peak determinations.

5.3.2 **Amplification Kit-Specific Artifacts**

5.3.2.1 **GlobalFiler™**

5.3.2.1.1 Reproducible kit-specific artifacts for GlobalFiler™ have been reported by the manufacturer and provided in the ThermoFisher Scientific Technical Note.

5.3.2.1.2 Additional reproducible kit-specific artifacts for GlobalFiler™ are outlined below:

5.3.2.1.2.1 During the development validation, essentially no reproducible dye artifacts were observed within the kit’s read region of 74 to 444 nucleotides.

5.3.2.1.2.2 During the OSBI forensic biology units’ internal validation, an artifact at TH01 was identified that typically occurred at n –

10 to n – 12 relative to the true allele. The size of the artifact trends with the size of the amplicon but was around 90 RFU.

5.3.2.1.2.3 Other commonly observed artifacts include:

5.3.2.1.2.3.1 ~180bp in red dye at D5S818 at ~50 RFU; and

5.3.2.1.2.3.2 ~394bp (allele 26) in red dye at SE33 at ~60 RFU.

5.3.2.1.2.3.3 DNA dependent artifact peaks adjacent to spectral pull-up peaks in the yellow and red dye channels sized ~0.5-1.5bp larger than the adjacent pull-up peak.

5.3.2.1.3 If observed above threshold, analysts should rename these artifacts as "ART" in the GMID-X project. Analysts should document the known artifact location and that the artifact was renamed.

5.3.2.1.4 The technical reviewer must agree with all renamed artifacts.

5.3.2.2 **Yfiler™ Plus**

5.3.2.2.1 Reproducible kit-specific artifacts for YFiler™ Plus have been reported by the manufacturer and provided in the ThermoFisher Scientific Technical Note.

5.3.2.2.2 Additional reproducible kit-specific artifacts for YFiler™ Plus are outlined below:

5.3.2.2.2.1 The incidence of minus and plus stutter is more significant at the tri-nucleotide repeat-containing loci DYS481 and DYS392.

5.3.2.2.2.2 Reproducible non-calling region artifacts observed in the OSBI forensic biology units' internal validation included:

5.3.2.2.2.2.1 90-91bp in blue dye (generally below 50 RFU);

5.3.2.2.2.2.2 ~234-235bp in green dye (generally below 50 RFU);

5.3.2.2.2.2.3 -5bp and -10bp at DYS391 in green dye;

5.3.2.2.2.2.4 ~70bp in green dye outside VIC read region (does not impact interpretation);

5.3.2.2.2.2.5 +6bp at DYS392 in yellow dye;

5.3.2.2.2.2.6 ~91-96bp, ~229-230bp, and ~234-235bp in yellow dye (generally below 50 RFU);

5.3.2.2.2.2.7 -5bp and -8bp at DYS437 in red dye;

5.3.2.2.2.2.8 Noisy baseline and multiple peaks at DYS385 in red dye, in addition to the peak outside the calling region at ~413 RFU with excess female DNA present in the sample;

5.3.2.2.2.2.9 +4bp at DYF387S1 in purple dye;

5.3.2.2.2.2.10 213-214bp in purple dye (generally below 50 RFU); and

5.3.2.2.2.2.11 ~348-349bp in blue dye (allele 17) in the positive control 007 DNA.

5.3.2.2.3 If observed above threshold, analysts should renamed these artifacts as "ART" in the GMID-X project. Analysts should document the known artifact location and that the artifact was renamed.

5.3.2.2.4 The technical reviewer must agree with all renamed artifacts.

5.3.3 Non-Specific Amplification Artifacts

5.3.3.1 Non-specific amplification products (artifacts) may occur due to excessive template amplification and/or the presence of co-extracted bacterial or microbial DNA. These artifacts may or may not have morphology similar to that of a true allele; however, they do not exhibit the presence of the characteristic stutter peak that accompanies the amplification of true human DNA.

5.3.3.2 If a non-specific amplification artifact is suspected, the sample type and the entirety of the profile should be evaluated to try to distinguish the artifact. The sample should be re-amplified, if possible.

5.3.3.2.1 If the peak could be the result of bacterial or microbial DNA present in the sample extract, re-amplification may not resolve the issue. However, the peak height of the non-specific amplification artifact may not be reproducible when re-amplified as would be expected with true, non-stochastic, human DNA.

5.3.3.3 These artifacts will be handled in consultation with the technical manager and/or Lead Analyst.

5.3.4 Analytical Artifacts

5.3.4.1 Instrument Spikes

5.3.4.1.1 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 reinjected to obtain a clean electropherogram.

5.3.4.1.2 Analysts will rename called spikes as "SP" in the GMID-X project if not already done so by the software.

5.3.4.1.3 The technical reviewer must agree with all spike determinations made by the analyst.

5.3.4.2 **Raised Baseline**

5.3.4.2.1 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 reinjected to obtain a clean electropherogram.

5.3.5 **Instrument Limitations**

5.3.5.1 **Off-scale Peaks (Oversaturation)**

5.3.5.1.1 An examination of the Oversaturation/Off-scale (OS) PQV in the 'Genotypes' tab is acceptable for determining whether a peak is off-scale. All samples will be evaluated for oversaturation/off-scale data and examined closer if the PQV is anything other than passing.

5.3.5.1.2 If the off-scale peak is not causing any interpretation problems, the injection may be used as long as off-scale peak(s) is/are documented.

5.3.5.1.3 If the off-scale peak is causing any interpretation problems, the analyst should attempt to alleviate the problem by reinjecting the sample.

5.3.5.1.4 If the off-scale peak is causing any interpretation problems and the reinjection resulted in the loss of allelic information, the analyst shall report the most informative profile.

5.3.5.1.5 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 the location.

5.3.5.1.6 Depending on the type or level of interpretation problems observed from off-scale data, analysts might have to re-amplify the sample at a lower amplification target to obtain reportable results.

5.3.5.2 **Pull-Up**

5.3.5.2.1 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.

5.3.5.2.2 Analysts will rename confirmed pull-up peaks as "PU" in the GMID-X project.

5.3.5.2.3 The technical reviewer must agree with all pull-up determinations.

5.3.6 **Introduction into the Analysis Process**

5.3.6.1 **Dye Blobs**

5.3.6.1.1 Dye blobs observed above threshold outside of allele call ranges shall be deemed acceptable without reinjection.

5.3.6.1.2 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 reinjected to obtain a clean electropherogram.

5.3.6.1.3 Analysts will rename dye blobs as “DB” in the GMID-X project.

5.3.6.1.4 The technical reviewer must agree with all dye blob determinations.

5.4 Interpretation of Allelic Peaks

The evaluation of allelic peaks includes allele designations given by the analysis software (GMID-X) and consideration of peak heights and peak height ratios at each locus and across the entire profile.

5.4.1 Peak Height Ratios

5.4.1.1 When a locus displays two alleles in a single source sample, the allele with the lower peak height generally displays at least 60% of the peak height of the higher peak at the locus.

5.4.1.1.1 For GlobalFiler™, the peak height ratio should generally be above 60%; however, exceptions can be CSF1PO, TPOX, D18S51, FGA, D7S820, SE33 and D2S1338 and these should have a ratio above 40%.

5.4.1.2 In a mixture profile, the relative peak heights may be in any relative contribution.

5.4.1.3 Peak height balance is affected by the amount and quality of template DNA available, so lower template samples may experience peak height balance less than these general percentages.

5.4.1.4 Regardless of peak height, peaks determined to be the result of stutter, pull-up, minus A, elevated baseline, spikes, or background will not be designated as true alleles.

5.4.1.5 The technical reviewer must agree with determinations outside these criteria. If there is discrepancy, the technical manager and/or Lead Analyst should be consulted.

5.4.2 Off-Ladder Alleles

5.4.2.1 Types of Off-Ladder Alleles

5.4.2.1.1 **Standard Alleles**: Alleles that fall **outside** the range of the ladder alleles. These alleles are smaller than the smallest allele of the ladder or larger than the largest allele of the ladder at a locus. The GMID-X software typically labels these alleles as “OL” when within the marker range of the locus. However, occasionally, an apparent allelic peak may be observed outside of or in between two loci marker ranges, and the GMID-X software typically labels these alleles as “OMR”.

5.4.2.1.2 **Microvariant Alleles**: Alleles that fall **inside** the range of the smallest and largest ladder alleles at a locus. These microvariant alleles contain an incomplete number of repeats, such as X.1, X.2 or X.3, and are labeled as “OL” by the GMID-X software if not present in the allelic ladder or a virtual bin.

5.4.2.1.3 **Virtual Alleles**: Alleles assigned a label by the GMID-X software due to the presence of a bin even though the allele itself is not represented in the allelic ladder. No additional analysis is required for virtual alleles. The GMID-X allele call will be accepted and used.

5.4.2.2 Locus Designation

5.4.2.2.1 When an off-ladder allele is observed between loci, and one neighboring locus appears to be homozygous while the other is heterozygous, the off-ladder allele most likely is a sister allele paired with the locus that appears to display homozygosity. Other off-ladder allele designations between loci shall be handled on a case-by-case basis in consultation with the technical manager and/or Lead Analyst.

5.4.2.2.2 An off-ladder allele may sometimes be labeled in the wrong locus. These instances may be observed as false tri-alleles or have inconsistent peak heights. These situations will be handled on a case-by-case basis in consultation with the technical manager and/or Lead Analyst.

5.4.2.3 **Allele Designation**

5.4.2.3.1 Off-ladder alleles will be assigned an allele value according to the following:

5.4.2.3.1.1 Designation of the alleles should refer to the total number of full repeats.

5.4.2.3.1.2 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 nine complete repeats and one incomplete repeat of 3 base pairs.

5.4.2.3.2 Occasionally, due to a sample not satisfactorily aligning with the average bins calculated from the ladder(s), an allele may be labeled as off-ladder by the GMID-X software even though its actual allele designation is recognized within the ladder. If this happens, the analyst should analyze the sample in a separate project with a different combination of ladder(s) to try to reconcile the sample.

5.4.2.4 **Calculating Off-Ladder Alleles**

5.4.2.4.1 **Standard Alleles**: Determine the base pair size of the 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 the off-ladder allele designation. For example, in D3S1358 the smallest ladder allele is 96.75 base pairs, designated as “9.” The off-ladder allele is 88.81 base pairs. 96.75 – 88.81 is 8. The size repeat for D3S1358 is 4 base pairs. 8/4 = 2, 9 – 2 = 7. The off-ladder allele would be designated as “7.”

5.4.2.4.2 **Microvariant Alleles**: 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. If the peak falls before or after the last peak in the ladder, the calculation will be handled in consultation with the technical manager and/or Lead Analyst. 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 233.57 bases and the “36” allele of the D21S11 ladder is 231.64 bases, the peak in question shall be labeled as “36.2” because the difference is 2.07, or 2 bases in length.

5.4.2.4.3 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.

5.4.2.4.4 All calculations should be documented on the electropherograms with sizes labeled or in the technical notes of the case record.

5.4.2.5 **Reporting Off-Ladder Alleles**

5.4.2.5.1 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://strbase.nist.gov/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.

5.4.2.5.1.1 If such an allele has been observed in the population, the allele shall be accepted and reported as determined. The allele will be renamed in the GMID-X project as determined.

5.4.2.5.1.2 If the allele has not been observed but is present in multiple samples, the allele shall be accepted and reported as determined. The allele will be renamed in the GMID-X project as determined.

5.4.2.5.1.3 If the allele has not been observed and is only present in one sample, that sample will be re-amplified, if possible, and the allele shall be accepted and reported as determined upon verification of reproducibility by re-amplification. The allele will be renamed in the GMID-X project as determined. 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.

5.4.2.5.2 Those alleles that fall outside the ladder for any locus shall be reported as the determined allele value/< (smallest ladder peak) or > (largest 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").

5.4.2.5.2.1 Alleles that fall outside the ladder will be entered on the CODIS Data Entry Form as <X or >X, as applicable.

5.4.2.5.3 All verified microvariant alleles should have the reference documented in the technical notes or on the electropherograms.

5.4.3 **Trisomy**

5.4.3.1 If a profile is suspected to have trisomy (tri-allelic) at a locus, the sample should be re-amplified to verify this event, if possible. If the trisomy is observed in multiple samples, no further analysis is necessary.

5.4.3.2 The trisomy can be reported; however, the locus shall not be used in any single source genotype frequency calculations.

5.4.4 **Insertion/Deletion/Null Alleles**

5.4.4.1 In rare instances, an insertion/deletion close to the STR repeat region may result in discordant results between two separate amplification kits due to the use of differing primers for the locus. Null alleles may occur due to primer binding site mutations, and alleles fail to amplify due to a sequence difference in a primer binding site mutation. Reporting of these situations will be handled in consultation with the technical manager and/or Lead Analyst.

5.4.5 **Y-STR Duplication/Triplication**

5.4.5.1 Regions of the Y chromosome may be duplicated or triplicated in males causing more than one peak at a locus. Duplicated loci generally have alleles with similar peak heights and are oftentimes only one repeat unit apart.

5.4.5.2 If a Y-STR haplotype appears otherwise single source and is suspected to have a duplication/triplication at a single locus where the two peaks are well balanced, the result can be reported; however, this locus shall not be used for statistical calculation.

5.4.5.3 If there are multiple loci with suspected duplication/triplication, consultation with the technical manager and/or Lead Analyst is required.

5.5 **Thresholds Used for Interpretation**

5.5.1 **Instrument Baseline**

5.5.1.1 Instrument baseline thresholds for all injection times per internal validation studies:

Instrument Baseline Thresholds (RFU)		
Dye Channel	**GlobalFiler™, Yfiler™ Plus**	**GlobalFiler™ Express, Yfiler™ Plus Direct Mode**
Blue	50	60
Green	50	60
Yellow	50	60
Red	50	60
Purple	50	60

5.5.1.2 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.

5.5.2 **Limit of Detection**

5.5.2.1 Limit of detection for amplification kits/injection times per internal validation studies:

GlobalFiler™ Limits of Detection (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	50	50
Green	50	50
Yellow	50	50
Red	50	50
Purple	50	50

Yfiler™ Plus Limits of Detection (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	50	75
Green	50	75
Yellow	50	75
Red	50	75
Purple	50	75

GlobalFiler™ Express Limits of Detection (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	60	60
Green	60	60
Yellow	60	60
Red	60	60
Purple	60	60

Yfiler™ Plus Direct Mode Limits of Detection (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	60	60
Green	60	60
Yellow	60	60
Red	60	60
Purple	60	60

5.5.2.2 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 (e.g. to confirm exclusion).

5.5.2.2.1 The technical reviewer must agree with the use of peaks below analytical threshold but above limit of detection. If there is disagreement, the technical manager will be consulted.

5.5.2.2.2 If a peak below analytical threshold but above limit of detection is believed to be the result of an allele, additional analysis may be attempted in an effort to bring as much data as possible above the analytical threshold (e.g. reinjection at an increased injection time, re-amplification with a higher target, additional analysis with another method, such as Y-STR analysis etc.). However, in some instances, additional analysis may not be practical due to higher peak heights of the other alleles in a sample or may not be necessary due to the amount of information present in the sample (e.g. if only 3 alleles are below analytical threshold).

5.5.2.3 Any further attempt(s) to assess peaks below the limit of detection will be up to the discretion of the analyst and the needs of the case.

5.5.3 **Analytical Threshold**

5.5.3.1 Analytical thresholds for amplification kits/injection times per internal validation studies:

GlobalFiler™ Analytical Thresholds (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	100	100
Green	100	100
Yellow	100	100
Red	100	100
Purple	100	100

Yfiler™ Plus Analytical Thresholds (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	100	150
Green	100	150
Yellow	100	150
Red	100	150
Purple	100	150

GlobalFiler™ Express Analytical Thresholds (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	120	120
Green	120	120
Yellow	120	120
Red	120	120
Purple	120	120

Yfiler™ Plus Direct Mode Analytical Thresholds (RFU)		
Dye Channel	**7s/15s**	**24s**
Blue	120	120
Green	120	120
Yellow	120	120
Red	120	120
Purple	120	120

5.5.3.2 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.

5.5.3.3 No peaks below the analytical threshold will be sized as alleles.

5.5.4 **Peak Stochastic Threshold**

5.5.4.1 Peak stochastic thresholds for all dye channels per internal validation studies:

Peak Stochastic Thresholds (RFU)				
	GlobalFiler™	**Yfiler™ Plus**	**GlobalFiler™ Express**	**Yfiler™ Plus Direct Mode**
7s/15s	530	300	250	250
24s	820	400	250	250

5.5.4.2 Samples may be classified as displaying stochastic effects if:

5.5.4.2.1 The profile has unpaired allelic peaks that have peak heights above the analytical threshold but below the stochastic threshold.

5.5.4.2.2 The profile has lower peak height ratios than would be expected between two paired allelic peaks.

5.5.4.2.3 The profile has extreme sloping.

5.5.4.2.4 The profile has allelic dropout.

5.5.4.3 Peaks observed below the peak stochastic threshold that do not appear to have a paired allelic peak cannot be assumed to be homozygous. There may be dropout of a sister allele.

5.5.4.4 Dropout of a potential paired allelic peak must be assumed for statistical calculations.

5.5.4.5 Peak height ratios of paired allelic peaks below peak stochastic threshold may also be lower than normal.

5.5.4.6 Any interpretation of mixtures with peaks below peak stochastic threshold should be done carefully and in the most conservative manner possible because if there are alleles present between the analytical and peak stochastic threshold, other alleles may have dropped out.

5.5.4.6.1 Y-STR mixtures with the highest peak below the peak stochastic threshold will be called *inconclusive* or in some instances may be interpreted with caution and in consultation with the technical manager and/or Lead Analyst.

5.5.4.7 Known samples with peaks below the peak stochastic threshold can be reported as genotype (e.g. 8,8) instead of phenotype (e.g. 8) as long as the technical reviewer agrees with this determination.

5.5.4.8 If possible, without causing uninterpretable results such as off-scale data or excessive pull-up, analysts may attempt to re-amplify or re-extract a sample in order to obtain a profile with all alleles above peak stochastic threshold. The potential for statistical calculations for any inclusion and CODIS eligibility should be considered when evaluating the need for additional analysis, as it may or may not be necessary based on the amount of information present in the sample.

5.6 Interpretation of Profiles (Types of Profiles)

5.6.1 General Considerations

5.6.1.1 Any assumptions made during interpretation shall be documented in the technical notes of the case record (e.g. intimate sample, number of contributors, presence of a known contributor, etc.).

5.6.1.2 When performing interpretations (and comparisons), keep in mind the potential for allele sharing and the additive effects of such sharing; this can affect not only allele peak heights but also stutter.

5.6.1.3 DNA typing results and suitability for statistical calculations for forensic unknown samples should be verified and interpreted to the extent reasonably possible.

5.6.1.4 If any profile has resolution issues or any other abnormality, the analyst will consult with the technical manager and/or Lead Analyst before reporting the data.

5.6.1.5 Non-paternity cases dealing with relatedness should be interpreted with extreme caution and in consultation with the technical manager.

5.6.2 Degradation

5.6.2.1 If a sample is degraded, higher molecular weight alleles may not amplify due to preferential degradation of larger alleles.

5.6.3 Allelic (Locus) Dropout

5.6.3.1 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, which may result in allelic (locus) dropout. 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).

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

5.6.3.2.1 There also appears to be allelic dropout occurring at loci with smaller base pair ranges (e.g. if a single peak at D21S11 is above peak stochastic threshold, it may be designated as partial if there is also

dropout occurring at D3S1358, Amelogenin, D8S1179, D2S441, D22S1045 or D10S1248).

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

5.6.4 **Samples That Appear to Be from One Source (Single Source Interpretation)**

5.6.4.1 **Single Source Profiles with All Peaks above Peak Stochastic Threshold**

5.6.4.1.1 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 peak stochastic threshold.

5.6.4.1.2 Likewise, if only one allele is detected at a locus and the allele is above the peak stochastic threshold, the analyst can be confident that there is no allelic dropout.

5.6.4.2 **Single Source Profiles with Peaks below Peak Stochastic Threshold**

5.6.4.2.1 If the sample appears to be from a single source and only one allele is detected at a locus that is below the peak stochastic threshold, the analyst may not be confident that the DNA profile is complete at that locus.

5.6.4.2.2 If the sample is a known reference sample and only one homozygous locus is below peak 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.

5.6.4.2.3 In such instances where there is not confidence that the profile is complete, the analyst must indicate that this single peak is below the peak stochastic threshold and appropriate statistical calculations will be performed on this locus using OSBI STATS with dropout marked “yes.”

5.6.4.2.4 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 peak stochastic threshold), the reasoning must be reflected in the technical notes and agreed on by the technical reviewer.

5.6.4.3 **Partial Single Source Profiles/Minimum Number of Loci for Interpretation**

5.6.4.3.1 Partial single source profiles must have interpretable results at a minimum of four loci for interpretation.

5.6.4.3.2 **Uninterpretable data:** If the genetic information detected in a partial profile is too minimal (i.e. < 4 loci), the profile will be deemed *inconclusive* or *not suitable for interpretation at this time*.

5.6.4.3.3 **Uninterpretable data:** Partial single source profiles with results at more than four loci may also be deemed *inconclusive* or *not suitable for interpretation at this time* due to the partiality of a profile (e.g. significant dropout observed despite results at > 4 loci).

5.6.5 **Samples That Appear to Be from More Than One Source (Mixture Interpretation)**[29]

5.6.5.1 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.

5.6.5.2 Based on the results of the OSBI in-house mixture study of three-, four-, and five-person mixtures, analysts will be allowed to interpret and report multi-person DNA mixture profiles consisting of a maximum count of six alleles at a single locus. 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 (see below).

5.6.5.3 **Assessing Number of Contributors to a Mixture**
Based on the OSBI in-house mixture study, analysts cannot accurately identify the number of contributors to a mixture. Instead, analysts will interpret DNA mixture profiles as a whole based on the number of peaks present at each locus to determine the *minimum* number of contributors to a mixture.

5.6.5.3.1 Peaks below analytical threshold but above the limit of detection will be considered toward the maximum allele count at a locus.

5.6.5.3.2 Analysts may use discretion in considering peaks filtered as stutter at a locus when considering the maximum allele count at a locus as long as their considerations are documented in the technical notes and the technical reviewer agrees.

5.6.5.3.3 Analysts can make a statement about the minimum number of contributors to a mixture profile in their technical notes (e.g. at least three contributors for a profile with five peaks at one or more loci). This information may also be included in the report.

5.6.5.4 **Deducing Potential Contributors to Mixtures**

5.6.5.4.1 Epithelial (non-sperm) and sperm fractions from the same item can be used as an aid during the interpretation process. For example, a single source contributor of one fraction of a differentially extracted sample may be used as a “known” to help interpret the other associated mixed fraction profile.

5.6.5.4.2 For mixture results from differential extracts or other samples when one of the contributors to a mixture is known, such as the donor of an intimate sample, the DNA profile of the unknown contributor may be inferred by subtracting out the contribution of the known donor from the mixture profile in order to facilitate identification of foreign alleles during data interpretation. A similar instance can arise when another known individual, such as a 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.

5.6.5.4.2.1 The DNA profile from an intimate item documented as collected from a known individual may be deemed as a known contributor.

[29] Reference “OSBI Mixture Flowchart” for guidance, if necessary.

5.6.5.4.2.2 When appropriate, the DNA profile of a documented known consensual sexual partner may be deemed as a known contributor.

5.6.5.5 If possible, analysts will determine if a mixture profile is major/minor or indistinguishable, as outlined below:

5.6.5.5.1 **Major/Minor Mixture Profiles (Separation of Contributors)** A mixture where the peak heights are such that a distinct major contributor can be determined. Any detected homozygous allele(s) of the major component is/are above the peak stochastic threshold. The detected alleles of the minor contributor may be above or below peak stochastic threshold.

5.6.5.5.1.1 If the "major" profile is (at each locus) neither clearly homozygous with all allelic peaks above the peak stochastic threshold nor has at least one allelic peak attributed to the major profile above the peak stochastic threshold if the locus is heterozygous, the technical reviewer must agree with the determination of "major/minor" for the DNA profile.

5.6.5.5.1.2 If the entirety of the major component is below peak stochastic threshold, technical manager approval must be obtained.

5.6.5.5.1.3 Although rare, there is the possibility of obtaining a result with an indistinguishable mixture in the major component with minor component alleles present. Profiles with this type of result will be interpreted in consultation with the technical manager and/or Lead Analyst.

5.6.5.5.1.4 Analysts should determine which apparently homozygous peaks of the minor component are below peak stochastic threshold and therefore might not fully represent the minor component.

5.6.5.5.1.5 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 peak stochastic threshold. It must be considered that the true minor component may not be fully represented.

5.6.5.5.1.6 If the minor component alleles are all above peak stochastic threshold, the minor component can be considered fully represented. However, in OSBI STATS, "Possible Dropout" will be marked "Yes" to account for the possibility of shared alleles with the major component for apparent single source minor components.

5.6.5.5.1.7 **Uninterpretable data:** For major/minor mixtures with seven or more alleles, or the presence of seven or more potential allele peaks above limit of detection (including both major and minor components) at one or more loci, the minor

component will be deemed *uninterpretable at this time* and will not be used for comparison purposes.

5.6.5.5.2 **Indistinguishable Mixture Profiles with All Peaks above Peak Stochastic Threshold**
A mixture where the peak heights are such that a distinct major contributor cannot be determined and all detected alleles are above peak stochastic threshold.

5.6.5.5.2.1 All alleles are detected, and it is reasonable to assume no dropout due to all alleles being above peak stochastic threshold.

5.6.5.5.2.2 **Uninterpretable data:** Indistinguishable mixtures with seven or more alleles, or the presence of seven or more potential allele peaks above limit of detection, at a single locus will be deemed *not suitable for interpretation* due to their high levels of complexity. Peaks below the analytical threshold but above the limit of detection are to be considered when making this determination.

5.6.5.5.3 **Indistinguishable Mixture Profiles with Peaks below Peak Stochastic Threshold**
A 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 peak stochastic threshold.

5.6.5.5.3.1 Indistinguishable mixtures with alleles present below peak stochastic threshold will be treated as having potential for dropout at all loci for statistical purposes.

5.6.5.5.3.2 **Uninterpretable data:** Indistinguishable mixtures with seven or more alleles, or the presence of seven or more potential allele peaks above limit of detection, at a single locus will be deemed *not suitable for interpretation* due to their high levels of complexity. Peaks below the analytical threshold but above the limit of detection are to be considered when making this determination.

5.6.5.5.4 **Partial Mixture Profiles/Minimum Number of Loci for Interpretation**

5.6.5.5.4.1 Partial indistinguishable mixtures, including minor component mixtures of two or more individuals, must have interpretable results at a minimum of six loci for interpretation.

5.6.5.5.4.2 Single source minor components must have interpretable results at a minimum of four loci for interpretation.

5.6.5.5.4.3 **Uninterpretable data:** If the genetic information detected in a partial mixture profile is too minimal (i.e. < 6 loci), the profile will be deemed *inconclusive* or *not suitable for interpretation at this time*.

5.6.5.5.4.4 **Uninterpretable data:** Partial mixture profiles with results at more loci than these limits may also be deemed *inconclusive* based upon complexity.

5.6.6 **Inconclusive Profile/Locus Determination (Criteria for Uninterpretable Data)**

5.6.6.1 When insufficient information is present in the profile to make a determination regarding interpretation, these profiles will be deemed *inconclusive* due to them being *not suitable for interpretation at this time* (i.e. uninterpretable). This determination could be due to the partiality of a profile or the extreme complexity of a profile.

5.6.6.2 Some technical issues may prevent the complete interpretation of a DNA profile or a locus in a profile; these profiles will be deemed *inconclusive* due to them being *not suitable for interpretation at this time* (i.e. uninterpretable). Examples of such situations may include:

- Accurate allele designations not possible due to poor quality injection, the allelic ladder, or the sample itself
- Alleles that cannot be definitively assigned to a locus
- Controls for the sample did not perform properly
- Data potentially affected by contamination

5.6.6.3 The "inconclusive" determination may also include the determination of suitability of loci for use in statistical calculations.

5.6.7 **Reinterpretation of Legacy Amplification Test Kit Data**

5.6.7.1 Analysts may use legacy data as it was interpreted by the original analyst for comparison purposes, CODIS entry, statistics, etc. When an analyst only uses the legacy data as-is, it is not considered reinterpretation. Examples that are not considered reinterpretation include:

5.6.7.1.1 The generation of a report for the comparison of two samples as a result of a CODIS high stringency match.

5.6.7.1.2 If the interpretation of the DNA profile from a forensic sample has previously been documented regarding the genotypes that would be allowed for possible contributors and the data is used as-is.

5.6.7.2 Assessing/evaluating (reevaluating) allele calls, genotype calls (to include potential allelic dropout), a change in the assumptions used, or removing alleles (or entire loci) from statistical estimates from legacy amplification test kit data, are all considered reinterpretation. Analysts will not reinterpret any data generated with a legacy amplification test kit if they do not satisfy the applicable requirements for reinterpretation (listed below).

5.6.7.2.1 When applicable, analysts must comply with the requirements for reinterpreting legacy data (listed below) when evaluating potential CODIS matches under certain circumstances. For example, moderate stringency matches between a candidate and target DNA profile where: 1) one or both of the DNA profile(s) originate from legacy data; and 2) the match involves comparisons of the original image(s) or electropherogram(s) to assess the match is considered reinterpretation.

5.6.7.3 **Analyst requirements for reinterpretation of legacy amplification test kit data:** In the event legacy data requires reinterpretation, if an analyst is sufficiently trained, has reviewed the validation and standard operating procedures for the appropriate application of analytical parameters (e.g. thresholds, peak height ratios), the analyst may review and reinterpret legacy data in accordance with the following:

5.6.7.3.1 If an analyst is currently qualified (trained and proficiency tested) in the amplification test kit, the analyst can reinterpret the data.

5.6.7.3.2 If an analyst is currently qualified in an amplification test kit (trained and proficiency tested), and the analyst was previously qualified (trained and proficiency tested) in the legacy amplification test kit, the analyst can reinterpret the legacy data if the analyst has been proficiency tested on the legacy test kit within the last two calendar years.

5.6.7.3.2.1 If an analyst has not been proficiency tested on a legacy test kit within the last two years, the technical manager must document and approve the completion of the analyst's review of the validation data and standard operating procedures of the legacy test kit.

5.6.7.3.2.2 If an analyst, who has completed the requirements to interpret legacy amplification test kit data, has not reviewed the required documents within the last two calendar years, the technical manager must document and approve the completion of the analyst's additional review.

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

5.6.7.3.3.1 If a previously qualified analyst is unavailable to train a currently qualified analyst on a legacy amplification test kit, the current analyst should not reinterpret legacy data.

5.6.7.3.3.2 If the analyst must perform legacy interpretation without training from a previously qualified analyst, then the analyst and technical manager will train in the legacy test kit interpretation protocols, review validation data and standard operating procedures. The training and interpretation competency must be completed by the analyst, and documented and approved by the technical manager. Documentation shall contain sufficient information for an auditor to confirm the analyst is qualified to reinterpret legacy data.

5.6.7.3.4 Technical reviewers of reinterpretations of legacy data will be held to the same requirements as the analyst reinterpreting the data, as listed above.

5.7 Profile Comparisons (Criteria for Formulating Inclusionary, Exclusionary & Inconclusive Conclusions)

5.7.1 General Considerations

5.7.1.1 DNA typing results and suitability for statistical calculations for forensic unknown samples should be verified and interpreted to the extent reasonably possible prior to performing comparisons to known reference samples and formulating inclusionary, exclusionary or inconclusive conclusions.

5.7.1.2 Questioned samples will be analyzed with all peaks being appropriately designated and interpreted before comparing to any known reference samples.

5.7.1.3 When formulating comparison conclusions, the overall quality of a sample (e.g. degradation, preferential amplification, inhibition, dropout, etc.), use of assumptions, or other guidance for interpretation shall be considered beyond solely the presence or absence of alleles.

5.7.1.4 The suitability for inclusion in the statistical calculation for each locus in a questioned profile must be made prior to comparison to known reference samples.

5.7.1.5 For an inclusionary conclusion, there must be a positive association between a questioned profile and a known reference sample (i.e. match or cannot be excluded). In general, when comparing a known reference sample to a questioned profile, all allelic information from the known reference sample must be present in the questioned profile at all loci with interpretable results.

5.7.1.6 For an exclusionary conclusion, no positive association exists between a questioned profile and a known reference sample (i.e. does not match or is excluded). Allelic information from the known reference sample is not present in the questioned profile at all or some loci.

5.7.1.7 For an inconclusive conclusion, a profile is not suitable for interpretation and no conclusions can be drawn. No comparisons will be made to profiles that have been deemed *inconclusive.*

5.7.1.7.1 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.

5.7.2 Partial Profiles (Single Source or Mixtures)

5.7.2.1 Some partial single source or mixture profiles may be insufficient for comparison, such as those that do not meet the required minimum number of loci for interpretation. These partial profiles may be used for exclusionary purposes only if the technical reviewer agrees.

5.7.2.2 When comparing profiles obtained between two different amplification kits, the required minimum number of loci for interpretation may be affected by non-concordant loci between the differing kits if the known sample(s) will not/cannot be analyzed with the same amplification kit.

5.7.3 **Mixture Profiles**[30]

5.7.3.1 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 with interpretable results in order to report the comparison as an inclusion.

5.7.3.2 The results of a comparison may occasionally be deemed an inclusion even if some alleles in the known reference sample are not present in the questioned mixture profile. In order to report an inclusion with some alleles not present in the mixture profile, the following criteria must be met:[31]

5.7.3.2.1 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.

5.7.3.2.2 For a partial mixture profile with results at eight or fewer loci, there must not be any more than one allele at a maximum of one locus where an allele from the known reference sample is not present in the mixture.

5.7.3.2.3 For a partial mixture profile with results at nine or more loci, there must not be any more than one allele at a maximum of two loci where an allele from the known reference sample is not present in the mixture.

5.7.3.2.4 For both complete and partial mixture profiles, there must be a scientifically supported reason (e.g. degradation, inhibition) that explains the absence of the allele(s).

5.7.3.2.5 If an inclusion is reported with some alleles not present in the mixture profile and any one of the above criteria is met, the comparison reasoning must be documented in the technical notes, DNA profile table, or another document, such as an email or a separate set of “comparison” technical notes, and included within the portfolio.

5.7.3.2.6 For both complete and partial mixture profiles, the technical reviewer must agree with the inclusion if any allele(s) in the known reference sample are not present in the mixture profile.

5.7.3.3 Some mixture profiles (minor components or indistinguishable mixtures) may be insufficient for comparison, such as those that do not meet the required minimum number of loci for interpretation or are too complex for interpretation. These mixture profiles may be used for exclusionary purposes only if the technical reviewer agrees.

Attachment(s):
None

[30] Reference “OSBI Mixture Flowchart” for guidance, if necessary.

[31] An exception to the required criteria are differential samples where the epithelial and/or sperm fraction have carryover and based on the opposing fraction result, the inclusion of a known reference sample is scientifically supported. This reasoning must be documented in the technical notes, DNA profile table, or another document, such as an email or a separate set of “comparison” technical notes, and included within the portfolio. The technical reviewer must also agree with the determination.

Reference(s):
ThermoFisher Scientific Technical Note for Artifacts Identified Post-Developmental Validation: GlobalFiler™ PCR Amplification Kit

ThermoFisher Scientific Technical Note for Artifacts Identified Post-Developmental Validation: Yfiler Plus™ PCR Amplification Kit

OSBI Mixture Flowchart

OSBI CODIS Unit Policy Manual

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories

SWGDAM Clarification of the Reinterpretation of Data Typed with Legacy Amplification Test Kits

OSBI Forensic Biology Units' Training Manual

CASE_6 DNA STATISTICAL ANALYSIS GUIDELINES (↑ Table of Contents)

Quick links:

6.1 Statistical analysis will be performed for all inclusionary, probative samples (i.e. reported inclusionary results), with the exception of intimate samples (if desired), to provide weight to the match/non-exclusion inclusionary statement.

6.1.1 When a full/complete single source questioned profile is obtained but the known reference sample exhibits a partial profile, statistics for the comparison will include only those alleles in the questioned profile at the loci corresponding to those that have results in the known reference profile in order to not overstate the strength of the inclusion since the statistic is provided in order to give a weight to the match or inclusion.

6.1.2 When profiles obtained with different amplification kits are compared, the statistics, if applicable, will be based on the concordant loci between the two amplification kits.

6.1.3 Uninterpretable data, such as loci deemed *inconclusive*, shall not be included in statistical calculations.

6.2 Statistics are not required for exclusionary conclusions, inconclusive/uninterpretable results or comparisons between multiple questioned samples with no comparison to a known reference sample.

6.3 **Autosomal STR Statistics**

6.3.1 **OSBI STATS**

6.3.1.1 STR statistics will be calculated using the OSBI forensic biology units' in-house Excel spreadsheet: "OSBI STATS" (**CASE_OSBI_STATS_v2.2**), which utilizes the allele frequencies from the National Institute of Standards and Technology (NIST) database at http://www.cstl.nist.gov/div831/strbase/NISTpop.htm.

6.3.1.2 Analysts shall refer to the OSBI Stats Guide (**CASE_OSBI_STATS_v2.2_Guide**) for guidance when using OSBI STATS to calculate statistics including, but not limited to, the approaches to performing statistical calculations.

6.3.1.3 Analysts should not save copies of OSBI STATS to their desktop computer with alternate settings that differ from the template provided in this policy.

6.3.2 **Single Source Profiles**

6.3.2.1 The random match probability (RMP) of a given genetic profile shall be estimated using OSBI STATS.

6.3.2.2 If a sample appears to be from a single source and two alleles are observed at a locus, regardless of the peak stochastic threshold, or if a homozygous peak is observed above the peak stochastic threshold, the analyst can be confident that the DNA profile at that locus is complete. Therefore, these loci may be used for statistical calculations with no dropout indicated in OSBI STATS.

6.3.2.3 If a sample appears to be from a single source and only one allele is detected at a locus and the zygosity is in question (i.e. 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 peak stochastic threshold by making an indication of this observation on the DNA profile table (e.g. bold/italicize the allele in the table). Depending on analyst discretion and interpretation, which is to be clearly articulated in the analyst's technical notes, for these profiles the following options exist:

6.3.2.3.1 The formula $2p - p^2$ may be used to address this situation without double-counting the proportion of homozygotes in the population.[32] This calculation will be performed using OSBI STATS and indicating dropout.

6.3.2.3.2 The analyst may choose to perform no statistical calculations on such loci.[33]

6.3.2.3.2.1 Loci in this category may still be useful for exclusionary purposes in this circumstance.

6.3.3 **Major Components of Mixture Profiles**
The OSBI STATS major component or single source calculation shall be used for the major component of a mixture.

6.3.4 **Minor Components of Mixture Profiles**

6.3.4.1 The OSBI STATS 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.

6.3.4.2 For any apparently homozygous allelic peaks in the minor component, regardless of whether they are above or below the peak stochastic threshold, the formula $2p - p^2$ will be used to account for potential allele sharing with the major component.

6.3.4.3 If any apparently homozygous allelic peaks are below the peak stochastic threshold, the minor component may or may not be suitable for interpretation. Depending on analyst discretion and interpretation, which is to be clearly articulated in the analyst's technical notes, for these profiles the following options exist:

[32] Per 4A.6 of the *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*, approved January 12, 2017.

[33] Per 3.4.5 & 4A.6 of the *SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories*, approved January 12, 2017

6.3.4.3.1 The formula $2p - p^2$ may be used to address this situation without double-counting the proportion of homozygotes in the population. This calculation will be performed using OSBI STATS and indicating dropout.

6.3.4.3.2 The analyst may choose to perform no statistical calculations on such loci.

6.3.4.3.2.1 Loci in this category may still be useful for exclusionary purposes in this circumstance. For example, if all loci have apparent homozygous peaks below the peak stochastic threshold in the minor component.

6.3.5 Indistinguishable Mixture Profiles

6.3.5.1 For an indistinguishable mixture where all detected alleles in the mixture are above peak stochastic threshold, it is reasonable to assume no dropout and these loci may be used for statistical calculations with no dropout indicated in OSBI STATS to calculate the probability of inclusion.

6.3.5.2 To calculate the probability that a randomly selected person would be included as a contributor to the mixture that has loci with alleles below the peak stochastic threshold, the analyst will use the formula $2p - p^2$ to address this situation without double-counting the proportion of homozygotes in the population. Indistinguishable mixtures with alleles present below peak stochastic threshold will be treated as having potential for dropout at all loci for statistical purposes. This calculation will be performed using OSBI STATS and indicating dropout.

6.3.6 Partial Profiles (Single Source or Mixtures)

6.3.6.1 Profiles that are partial shall be calculated using the appropriate formula (single source or mixture) at the loci where all alleles are determined to be present above the peak stochastic threshold, or the formula $2p - p^2$ may be used if there are loci with alleles below the peak stochastic threshold 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.

6.3.7 Family Relatedness Statistics

6.3.7.1 Single source profiles, including single source major components, are suitable for family relatedness statistical calculations. Indistinguishable mixtures and minor component profiles cannot be interpreted for the purposes of paternity and are not suitable for family relatedness statistical calculations.

6.3.7.2 Likelihood Ratios (LR) will be used for family relatedness statistical calculations using OSBI STATS (when possible). If the calculations fall outside of the capabilities of OSBI STATS, a hand calculation may be used, in consultation with the technical manager, or the requesting agency may be referred to an alternate outside agency to perform the calculations.

6.3.7.3 The appropriate formula will be used for each locus given the circumstances of the case (i.e. Parentage, Alleged Parent [Known Mother with Alleged Father] or Parentage, Alleged Child [Unidentified Remains with Alleged Parents]). A combined likelihood ratio ($LR_{cumulative}$) will be determined by multiplying all the likelihood ratios calculated for each locus.

6.3.7.3.1 **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.

6.3.7.3.2 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.

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

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

6.4 Male-Specific Y-STR Statistics

6.4.1 Y-Chromosome Haplotype Reference Database (YHRD)

Y-STR statistics will be calculated using the YHRD website (www.yhrd.org), which accepts the Y-STR allelic data and calculates the estimated frequency of the profile based on the data present in the database using the counting method.

6.4.2 Single-Source & Major Components of Mixture Profiles (Including Partial Profiles)

6.4.2.1 Statistics will be calculated for partial or full (complete) single source Y-STR profiles and for the major components of Y-STR mixtures that satisfy interpretation criteria in CASE_5.

6.4.2.2 Statistics will be calculated using the Dataset = Kit (“masked search”) method by selecting the following on the YHRD website:

6.4.2.2.1 Select “Estimate Frequency” in the menu bar at the top of the page
6.4.2.2.2 Select the “Manually enter haplotype(s)” button
6.4.2.2.3 Select Dataset: “Y27”
6.4.2.2.4 Select Kit: “Yfiler Plus”
6.4.2.2.5 Select the “Search” button
6.4.2.2.6 Select the “+Add feature to this Report” button
6.4.2.2.7 Select “National Database (with Subpopulations, 2014 SWGDAM-compliant)”

6.4.2.3 Profiles with only one allele called at a multi-copy locus (DYS385/DYF387S1) that is above analytical threshold but below peak stochastic threshold will not be included in the statistical calculation.

6.4.2.4 Profiles with two alleles called at a multi-copy locus (DYS385/DYF387S1) can be included in the statistical calculation regardless of whether the alleles are above or below the peak stochastic threshold because the presence of both alleles indicates that dropout has not occurred.

6.4.2.5 Profiles with one allele called at a multi-copy locus that is above peak stochastic threshold (with no indication of a second allele) can be included in the statistical calculation and will be entered as N,N (e.g. 35,35).

6.4.2.6 See the User’s Guide on the website above for additional information and updates.

6.4.3 Minor Components & Indistinguishable Mixture Profiles

Based on current SWGDAM guidelines, no statistical analysis shall be performed for Y-STR minor components or Y-STR indistinguishable mixtures.

Attachment(s):
Forensic Biology Units' Policy Manual **CASE_OSBI_STATS_v2.2**

Forensic Biology Units' Policy Manual **CASE_OSBI_STATS_v2.2_Guide**

Reference(s):
2014 SWGDAM Compliant YHRD User's Guide

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

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OSBI Forensic Biology Units' Training Manual

CASE_7 REPORT WORDING (↑ Table of Contents)

Quick links:

7.1 General Requirements

7.1.1 Results must be reported for all evidence tested.

7.1.1.1 For any test results that are not consistent for an item (e.g. one vaginal swab p30 positive and another vaginal swab p30 negative), the report shall include all results and clearly describe which item(s) had which result(s).

7.1.1.2 In the event controls fail and tests cannot be repeated, report wording should indicate results are inconclusive due to control failure.

7.1.1.3 If inconclusive results are obtained or no conclusions are drawn, the reason for this determination shall be included in the report wording.

7.1.2 Any evidence received by the reporting analyst but not tested will be listed in the report and identified as not analyzed.

7.1.3 Reports will clearly reflect any evidence created or collected by the reporting analyst and preserved that could be used for future testing. For example, if 7 soda cans are received and each swabbed for DNA but only 2 are analyzed for DNA, the report should reflect that each can was swabbed for DNA and 2 of the 7 swabs [items X & Y] were analyzed. However, items that are created for testing purposes that are tested and reported do not need to be specifically listed (e.g. cell search slides).

7.1.4 Based on accreditation requirements, when sample selection is used, wording must be present in the report to indicate that the results apply only to the portions of the item(s) tested.

7.1.5 The disposition for all evidence submitted for analysis and any additional associated derivative evidence removed from an item of evidence (e.g. swab of an item) shall be included in the report.

7.2 Recommended Report Wording

Although the intent of this section is to standardize report wording, 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 modified, combined, etc., as necessary, based on case circumstances, analyst preference, or for clarification of results. Final discretion regarding interpretation is left to the analyst, in consultation with the technical manager and/or Lead Analyst, if needed.

7.2.1 Blood Analysis

7.2.1.1 Hemastix® &/or Takayama

7.2.1.1.1 If visual inspection showed no blood-like staining, so no chemical testing was performed: *No blood-like staining was observed on…*

7.2.1.1.2 Use the following table to find the combination of tests performed and results obtained. Then look at the number indicated and use it 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.*

Blood was not detected.

(2) *Blood was detected.*

(3) *Presumptive testing indicated the (possible) presence of blood; however, the (actual) presence of blood could not be confirmed.*

(4) *Presumptive testing indicated 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.*

7.2.1.1.2.1 Analysts 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 provides the number of areas/swabs/hairs tested on/from an item and the results. This wording can be applied to any applicable examples provided in this policy.

7.2.1.2 **RSID™-Blood**

7.2.1.2.1 Positive result: *Human blood was detected.*

7.2.1.2.2 Negative result: *Human blood was not detected.*

7.2.1.2.2.1 Negative RSID™-Blood result with positive Takayama result: *Blood was detected; however, the blood is of non-human origin.*

7.2.1.2.2.2 Negative RSID™-Blood result with positive Hemastix® result: *Presumptive testing indicated the presence of blood; however, the (actual) presence of human blood could not be confirmed.*

7.2.2 **Sexual Assault Analysis**

7.2.2.1 **Crimescope (no further analysis)**
No staining consistent with seminal fluid was observed.

7.2.2.2 **AP Spot Test**

7.2.2.2.1 Positive result with spermatozoa negative and p30 negative results: *Acid phosphatase activity was detected. All other tests for seminal fluid were negative.*

7.2.2.2.2 Negative result: *No acid phosphatase activity was detected.*

7.2.2.3 **P30 &/or Microscopic Identification of Spermatozoa**

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

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) *Sperm(atozoa) were observed; however, p30 was not detected.*

Seminal fluid was detected based on the observation of sperm(atozoa); however, tests for the presence of p30 were negative.

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.

7.2.3 **Hair Analysis (Includes Debris/Fiber Collection)**

7.2.3.1 Report wording should include the number of hair selected for further DNA analysis.

7.2.3.2 **Macroscopic Hair Selection**

Human hairs were observed on Questioned Sample Description (item Q). They were macroscopically compared to a known hair sample from Known Sample Name (item K) in an attempt to determine the most suitable hairs for DNA analysis for the case. The hairs from Questioned Sample Description (item Q) (have/do not have) similar macroscopic characteristics to the known hair sample from Known Sample Name (item K). This/These hair(s) will (be/not be) forwarded for DNA analysis.

7.2.3.3 **Macroscopic Examination for Mitochondrial DNA Analysis**
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.*

7.2.3.4 **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.

7.2.3.5 **Microscopic Examination for 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.

7.2.3.6 **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.

7.2.3.7 **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.*

7.2.3.8 **Debris/Possible Hair/Possible Fiber Observation/Collection**
During the examination of Questioned Sample(s) Description(s) (item[s] Q, R, S…), debris/possible hair(s)/possible fiber(s) was/were observed [that may be suitable for additional testing] but not collected at this time. No further analysis was performed at this time.

Debris/Possible hair(s)/Possible fiber(s) was/were collected from Questioned Sample(s) Description(s) (item[s] Q, R, S…) but was/were not analyzed at this time.

7.2.4 **Y-Screen Assay (Direct to DNA Approach)**

7.2.4.1 Results for all samples where the Y-screen assay was performed must be reported, and each report will contain the following: *Deoxyribonucleic acid (DNA) from Questioned Sample Description (item Q) was extracted and quantitated to screen for the presence of [human] male DNA.*

7.2.4.2 Positive result with no further testing (pending additional DNA analysis): *[Human] male DNA was indicated/detected. No further analysis was performed at this time. (Items will be forwarded for additional DNA analysis.)*

7.2.4.3 Positive result with additional DNA testing (satisfying 7.2.5.6.1): *[Human] male DNA was indicated/detected. Additional DNA was isolated from the item(s) (or list items) and characterized through the polymerase chain reaction (PCR) using the short tandem repeat (STR) GlobalFiler™ amplification kit and/or male-specific short tandem repeat (Y-STR) Yfiler™ Plus amplification kit.*

7.2.4.4 Negative result: *[Human] male DNA was not indicated/detected.*

7.2.4.5 Inconclusive result: *The screening results were inconclusive due to the possible presence of an unknown substance interfering with the test. No conclusions could be drawn regarding the presence or absence of [human] male DNA on this/these item(s).*

7.2.4.6 Sample(s) with associated control failure: *The results obtained from the Questioned Sample Description (item Q) could not be interpreted due to technical limitations. No conclusions could be drawn regarding the presence or absence of [human] male DNA on this/these item(s).*

7.2.5 **DNA Analysis**

7.2.5.1 Results and/or conclusions for all samples where DNA analysis was attempted must be reported.

7.2.5.2 Samples taken from the same parent stain/swab may be reported as one profile from the analysis that provides the most genetic information.

7.2.5.3 If the same profile is obtained for multiple items and would result in the same statistical calculation, the items can be reported together so long as the results are clearly articulated. If a differing profile is obtained from items, the results shall be reported separately, even if the statistical results would be the same or if only exclusions are reported.

7.2.5.4 If the questioned sample and known sample are worked in different amplification kits, the term “match” cannot be used since differing loci were used for each samples’ analyses. The term “is consistent with” or “cannot be excluded” should be used for these inclusionary statements.

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

7.2.5.6 **Elements of a DNA Report**
Each DNA report will contain the following:

7.2.5.6.1 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) GlobalFiler™ amplification kit and/or male-specific short tandem repeat (Y-STR) Yfiler™ Plus amplification kit.*

7.2.5.6.2 An informative statement for differential extractions, if performed: *The analysis of item X included separating the item into two fractions/portions: an epithelial fraction/portion and a sperm fraction/portion.*

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

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

7.2.5.6.4 The type of profile identified (for questioned samples); analysts may elect to include biological/genetic sex (male/female) descriptors, if desired, understanding biological/genetic sex does not necessarily always impute phenotypic sex/gender identity, as applicable:

7.2.5.6.4.1 *No DNA profile was obtained.*

7.2.5.6.4.2 *A full/complete, single source DNA profile was obtained.*

7.2.5.6.4.3 *A mixture of DNA was obtained that was able to be separated into a major component and a minor component.*

The DNA profile obtained was a mixture that could be separated into a major component and a minor component.

7.2.5.6.4.4 *An indistinguishable mixture DNA profile was obtained.*

A DNA mixture/An indistinguishable DNA mixture [of at least X individuals] was obtained.

7.2.5.6.4.5 *A partial DNA profile was obtained.* (single source or mixture)

7.2.5.6.4.5.1 The report wording must reflect that a partial profile was obtained by either using the wording “partial profile” in the results statement or listing the number of loci at which the profile has been identified if fewer than the maximum number of loci amplified.

7.2.5.6.5 A statement of inclusion, exclusion, or inconclusive (see example statements provided at the end of this policy):

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

7.2.5.6.5.1.1 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 questioned profile matches.

7.2.5.6.5.1.2 Non-Exclusion (Cannot Be Excluded) Statement – applies to mixtures where profiles cannot be deduced to single genotypes at all loci and should indicate who cannot be excluded as a possible contributor. This statement also applies to single source profiles that exhibit possible incomplete genotypes at any locus.

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

7.2.5.6.5.2.1 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.

7.2.5.6.5.2.2 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 that exhibit possible incomplete genotypes at any locus.

7.2.5.6.5.3 **Inconclusive/Not Suitable for Interpretation Statements** The reporting that a profile is not suitable for interpretation and no conclusions can be drawn (i.e. inconclusive).

7.2.5.6.6 A statistical statement for all DNA inclusions, if applicable, excluding intimate samples, in order to report the weight of the match/non-exclusion statement. Statistics are not required for exclusionary conclusions, inconclusive/uninterpretable results, or comparisons between multiple questioned samples with no comparison to a known reference sample.

7.2.5.6.6.1 The statistical information shall be added as the final statement of the inclusion wording.

7.2.5.6.6.2 The statistical probability reported in forensic autosomal STR casework shall be the probability generated by OSBI STATS.

7.2.5.6.6.2.1 The most conservative statistic from the three most common ethnic/population groups in Oklahoma (Caucasian, African American, and Southwest Hispanics) shall be reported.

7.2.5.6.6.2.2 The number reported shall be no more than three significant figures (truncated).

7.2.5.6.6.3 The statistical results reported in forensic male-specific Y-STR casework shall be the observed value in the United States (Overall) using the “National Database (with Subpopulations) – United States” generated by the YHRD.

7.2.5.6.6.4 If statistics are not reported on an intimate sample, one of the following statements (based on profile type) must be added to the report wording for the item:
Due to the intimate nature of the sample, Known Sample Name (item K) is presumed to be the source of this DNA profile.

– or –

Due to the intimate nature of the sample, Known Sample Name (item K) is presumed to be a contributor to this DNA mixture.

7.2.5.6.6.5 Statistical calculations reported that have dropout indicated in OSBI STATS will include one of the following statements (based on profile type) as a footnote in the report and clearly reflect each item it applies to:
The statistical calculation performed accounts for potential missing genetic information (due to low level/quality of DNA present) and is a conservative estimate for the probability of selecting an unrelated individual at random from the population who could also be a potential donor/contributor to the profile obtained.

– or –

The statistical calculation performed for this item accounts for potential allele sharing between the major and minor components and is a conservative estimate for the probability of selecting an unrelated individual at random from the population who could also be a potential donor/contributor to the profile obtained.

7.2.5.6.6.6 Statistical calculations reported where a locus has not been included for statistics, in consultation with the technical manager and/or Lead Analyst, will use the following wording:
A DNA mixture [of at least X individuals] was obtained from Questioned Item Description (item Q) with genetic information available at N of X 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.

7.2.5.6.7 Any additional applicable information about the evidence including, but not limited to, disposition of evidence, including identifying when evidence is:

7.2.5.6.7.1 Returned to the submitting agency;

7.2.5.6.7.2 Retained by the laboratory;

7.2.5.6.7.3 Consumed;

7.2.5.6.7.4 Transferred between OSBI CSD Units, to include associated derivative evidence removed from an item (e.g. swab of an item); or

7.2.5.6.7.5 Any other wording to convey the status of the evidence at the time of reporting the DNA results;

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

7.2.5.7 **Examples of Results/Conclusions Statements & Other Statements**
The examples provided below are for inclusion, exclusion, no interpretive conclusions, inconclusive, and other report wording, and they are categorized under the situation that most commonly warrants them. These guidelines are not an

exhaustive list of all scenarios. The statistical statement wording has been included in the examples for clarity; this wording is not necessary for exclusionary statements.

7.2.5.7.1 **Single Source Profiles**

7.2.5.7.1.1 **Full/Complete Single Source Profile**
The DNA profile [from 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*** *the DNA profile from Known Sample Name (item K); he/she is* ***excluded*** *as a potential donor.*

7.2.5.7.1.1.1 **When a full/complete single source questioned profile is obtained but the known reference sample exhibits a partial profile:** If the known reference sample exhibits a partial profile (degraded) (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 reference sample.

The DNA profile [from Questioned Item Description (item Q)] ***is consistent*** *with the partial DNA profile 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 partial DNA profile is at least 1 in X.*

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

7.2.5.7.1.2 **Partial Single Source Profile**[34]
A partial single source profile where all alleles are accounted for by the known reference sample but up to one allele per locus present in the known does/does not appear in the questioned sample for scientifically supported reasons:

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

[34] Applicable wording may be used/modified for partial major components of major/minor mixture profiles, as necessary.

A partial DNA profile was obtained [from Questioned Item Description (item Q)] [with genetic information available at N of X 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 as least 1 in X.*

– or –

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

– or –

The DNA profile from Known Sample Name (item K) ***is consistent*** *with the partial DNA profile obtained [at N of X loci] from Questioned Item Description (item Q). The probability of selecting an unrelated individual at random from the population having this partial DNA profile is at least 1 in X.*

7.2.5.7.1.2.2 **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 potential donor 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 partial DNA profile is at least 1 in X.*

7.2.5.7.1.2.3 **If partial profile is insufficient for comparison due to limited information:** *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.*

7.2.5.7.1.2.4 **If many of the alleles are missing where an analyst is not confident in the match, or both alleles at a locus from the known reference sample are missing from the questioned sample:** *No conclusions can be made regarding comparison 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 excluded or imply any positive association with Known Sample Name (item K).

7.2.5.7.1.2.5 **General exclusion statement:** *Known Sample Name (item K) is **excluded** as a potential donor.*

7.2.5.7.2 **Major/Minor Mixture Profiles**

7.2.5.7.2.1 **Major/Minor Mixture Profile with Single Source Minor – No Dropout**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

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

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

*Known Sample Name (item K) is **excluded** as a potential donor of the minor component.*

7.2.5.7.2.2 **Major/Minor Mixture Profile with Single Source Minor – Dropout Indicated**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

*The DNA profile from the major component **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 the major component **does not match** the DNA profile from 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.*

7.2.5.7.2.3 **Major/Minor Mixture Profile with Minor >1 Person (Indistinguishable Mixture)**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

*The DNA profile from the major component **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 the major component **does not match** the DNA profile from 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 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.*

7.2.5.7.2.4 **Major/Minor Mixture Profile with Minor Insufficient for Comparison**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

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

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

7.2.5.7.2.5 **Major/Minor Mixture Profile with Minor Used for Exclusionary Purposes Only**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

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

*The minor component does not satisfy the laboratory's inclusionary reporting criteria; therefore, it may be used for exclusionary purposes. Based on the results, the Known Sample Name (item K) is **excluded** as a potential contributor*

7.2.5.7.2.6 **Major/Minor Mixture Profile with Major >1 Person (Indistinguishable Mixture)**

The DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

*The DNA profile from the major component is a 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 that could be a potential contributor to the major component is at least 1 in X.*

*Known Sample Name (item K) is **excluded** as a potential contributor to the major component.*

(For minor component, select the appropriate wording from above.)

7.2.5.7.3 **Indistinguishable Mixture Profiles**

7.2.5.7.3.1 **Indistinguishable Mixture Profile with No Dropout**

*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 potential contributor to the mixture is at least 1 in X.*

*Known Sample Name (item K) is **excluded** as a potential contributor.*

7.2.5.7.3.2 **Indistinguishable Mixture with Dropout Indicated**

*A 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 potential contributor. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to the mixture is at least 1 in X.*

*Known Sample Name (item K) is **excluded** as a potential contributor.*

7.2.5.7.3.3 **Partial Indistinguishable Mixtures**

7.2.5.7.3.3.1 **Partial indistinguishable mixture with dropout Indicated:** *A partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (item Q)] [with genetic information available at N of X locations analyzed.] 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 the mixture is at least 1 in X.*

Known Sample Name (item K) is ***excluded*** *as a potential contributor.*

7.2.5.7.3.3.2 **If many of the alleles are missing where an analyst is not confident in the inclusion:**
A partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (item Q)] [with genetic information available at N of X locations analyzed.] No conclusions can be made regarding the comparison 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. The partial mixture does not contain enough genetic information to either excluded or imply any positive association with Known Sample Name (item K).

– or –

A partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (item Q)] [with genetic information available at N of X locations analyzed.] 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 Questioned Sample Description (item Q) to Known Sample Name (item K). Known Sample Name (item K) can neither be included nor excluded as a potential contributor to Questioned Sample Description (item Q).

7.2.5.7.3.3.3 **Inconclusive partial indistinguishable mixture:** *An inconclusive partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (item Q)] [with genetic information at only N of X locations analyzed (or list loci if desired)]. There is not enough genetic information to either exclude or imply any positive association with Known Sample Name (item K).*

7.2.5.7.3.4 **Indistinguishable Mixtures with 7 or More Alleles/Potential Allele Peaks at a Locus**
A DNA mixture was obtained [from Questioned Item Description (item Q)]. Due to the complexity of the mixture, 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 Questioned Item Description [item Q].)

7.2.5.7.3.5 **Indistinguishable Mixtures Used for Exclusionary Purposes Only**
*A DNA mixture was obtained [from Questioned Item Description (item Q)]. However, it does not satisfy the laboratory's inclusionary reporting criteria; therefore, it may be used for exclusionary purposes. Based on the results, the Known Sample Name (item K) is **excluded** as a potential contributor.*

7.2.5.7.3.6 **Relatedness (Father, Mother & Child/Children)**
*A 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 potential contributor. The probability of selecting an unrelated individual at random from the population who could be a potential contributor to the 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 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.*

7.2.5.7.4 **Paternity Cases**

7.2.5.7.4.1 **When reporting standard inclusions/exclusions:**
*Alleged Father Sample Name (item AF) **cannot be excluded** with a ($Prob_{post}$) probability as the biological father of Child Sample Name (item C) given Mother Sample Name (item M) is the biological mother of the child.*

*Alleged Father Sample Name (item AF) is **excluded** as the biological father of Child Sample Name (item C) given*

Mother Sample Name (item M) is the biological mother of the child.

7.2.5.7.4.2 **If all paternal obligate allele are not present but could be accounted for by mutation, etc., the report wording (or a footnote) should recommend that the agency contact an appropriate parentage laboratory for further analyses:** *During DNA analysis, a result inconsistent with paternity was observed at X of N genetic locations analyzed; however, these results are not uncommon in paternity cases and are typically due to mutations that occur during the formation of sperm and egg, which are then passed onto offspring. Such mutations can lead to the false exclusion of a true parent if not properly evaluated. Your agency should consider contacting an appropriate parentage laboratory for further analysis. The frequency of the mutation observed has been accounted for in the statistical calculations reported in this report.*

7.2.5.7.4.3 **If an indistinguishable mixture is obtained from any tissue sample accepted for analysis:** *An indistinguishable DNA mixture was obtained from Item Description (item X). Indistinguishable mixtures obtained from samples in paternity cases cannot be interpreted at this laboratory. No further analysis was performed on this item at this time. Please contact an appropriate laboratory for analysis (interpretation and comparison) of this sample.*

7.2.5.7.5 **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 Names (items K…) do not account for all of the genetic information obtained from this DNA mixture.*

7.2.5.7.6 **Profiles with No Known Samples for Comparison**
A (partial/full/complete) (single source/mixture) profile was obtained from Questioned Sample Description (item Q) [at number of loci]. Please submit known samples for comparison purposes.

7.2.5.7.7 **Insufficient Profile (i.e. does not meet OSBI reporting requirements)**
The DNA profile obtained from Questioned Sample Description (item Q) is insufficient for comparison.

7.2.5.7.8 **No DNA Profile Obtained**
No DNA profile was obtained from Questioned Sample Description (item Q).

7.2.5.7.9 **Inconclusive Profiles**

7.2.5.7.9.1 **For DNA results where the genetic information is too limited to perform comparisons:** *Based on the limited genetic information obtained [from Questioned Item Description (item Q)], is not suitable for interpretation and no conclusions can be made at this time.*

7.2.5.7.9.2 **For DNA results where the genetic information is too complex for interpretation:** *Due to the complexity of the mixture obtained [from Questioned Item Description (item Q)], 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 Questioned Item Description [item Q].)*

7.2.5.7.9.3 **For comparisons where there is insufficient information in a questioned profile to exclude individual(s), but also insufficient information to conduct a statistical evaluation:**
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 Questioned Item Description (item Q). Known Sample Name (item K) can neither be included nor excluded as a contributor to Questioned Item Description (item Q).

– or –

An inconclusive partial DNA mixture [of at least X individuals] was obtained [from Questioned Item Description (item Q)] with genetic information at only N of X 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)

7.2.5.7.9.4 **For instances where technical issues, allele resolution issues, associated controls affected, results deemed not suitable for interpretation/inconclusive during interpretation even though reporting standards are met, etc.:** *An inconclusive DNA profile (or profile not suitable for interpretation) was obtained [from Questioned Item Description (item Q)] [at the A, B, C &… loci] due to [list reason].*

7.2.5.7.10 **Extracted DNA Not Amplified**

7.2.5.7.10.1 **Extraction only:** *DNA was isolated from Questioned/Known Sample Description (item Q/K). No further analysis was performed at this time.*

7.2.5.7.10.2 **Extraction and Quantitation:** *DNA from Questioned/Known Sample Description (item Q/K) was isolated and quantitated. Human DNA/human male DNA was/was not indicated/detected. No further analysis was performed at this time.*

7.2.5.7.11 **Miscellaneous**
Questioned Sample Description (item Q) was not profiled/typed [at this time].

Questioned Sample Description (item Q) was not analyzed [at this time].

7.2.5.7.12 **Sample(s) 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).

7.2.5.7.13 **Profiles Matching Staff**

7.2.5.7.13.1 **Single Source**: *An identified profile (profile consistent with OSBI personnel) was obtained from Questioned Sample Description (item Q) and the data was determined to be unreportable.*

7.2.5.7.13.2 **Mixture**: *DNA from more than one individual was obtained from Questioned Sample Description (item Q). An identified profile (profile consistent with OSBI personnel) was obtained in this sample and the data was determined to be unreportable. No further conclusions can be made regarding the DNA profile from this item.*

7.2.5.7.14 **Y-STR Profiles**

7.2.5.7.14.1 **Full/Complete Single Source Y-STR Profile**
*The Y-STR DNA profile [from Questioned Item Description (item Q)] **matches** the Y-STR DNA profile from Known Sample Name (item K). Therefore, Known Sample Name (item K) (and all his paternal male relatives) is **included** as a potential donor. Using a published Y-STR DNA population database, this Y-STR profile has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).*

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

7.2.5.7.14.1.1 **When a full/complete single source questioned profile is obtained but the known reference sample exhibits a partial profile:** If the known reference sample exhibits a partial profile (degraded) (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 reference sample.

*The Y-STR DNA profile [from Questioned Item Description (item Q)] **is consistent** with the partial Y-STR DNA profile from Known Sample Name (item K) [at X genetic locations yielding results]. Therefore, Known Sample Name (item K) (and all his paternal male relatives) is **included** as a potential donor. Using a published Y-STR DNA population database, this Y-STR profile*

has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).

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

7.2.5.7.14.2 **Partial Single Source Y-STR Profile**

7.2.5.7.14.2.1 **When all alleles from the known reference sample are represented in the questioned sample at all loci where results are obtained:**
A partial Y-STR DNA profile was obtained [from Questioned Item Description (item Q)] [with genetic information available at N of X locations analyzed]. Known Sample Name (item K) (and all his paternal male relatives) ***cannot be excluded*** *as a potential donor. Using a published Y-STR DNA population database, this partial Y-STR profile has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).*

– or –

The partial Y-STR DNA profile [from Questioned Item Description (item Q)] ***is consistent*** *with the Y-STR DNA profile from Known Sample Name (item K). Therefore, Known Sample Name (item K) (and all his paternal male relatives) is* ***included*** *as a potential donor. Using a published Y-STR DNA population database, this partial Y-STR profile has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).*

– or –

The DNA profile from Known Sample Name (item K) ***is consistent*** *with the partial Y-STR DNA profile obtained [at N of X loci] [from Questioned Item Description (item Q)]. Therefore, Known Sample Name (item K) (and all his paternal male relatives) is* ***included*** *as a potential donor. Using a published Y-STR DNA population database, this partial Y-STR profile has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).*

7.2.5.7.14.2.2 **If partial profile is insufficient for comparison:** *A partial Y-STR 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.

7.2.5.7.14.2.3 **If many of the alleles are missing where an analyst is not confident in the match or incomplete results:**
Due to the partial nature of the Y-STR DNA profile [from Questioned Sample Description (item Q)], it is insufficient for comparison. Known Sample Name (item K) can be neither included nor excluded from the partial Y-STR profile obtained.

– or –

No conclusions can be made regarding comparison 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 partial Y-STR profile does not contain enough genetic information to either exclude or imply any positive association with Known Sample Name (item K).

7.2.5.7.14.2.4 **General exclusion statement:** *Known Sample Name (item K) is **excluded** as a potential donor.*

7.2.5.7.14.3 **Y-STR Major Component Mixture Profile**[35]

The Y-STR DNA profile [from Questioned Sample Description (item Q)] was a mixture that could be separated into a major component and a minor component.

*The Y-STR DNA profile from the major component **matches** the Y-STR DNA profile from Known Sample Name (item K). Therefore, Known Sample Name (item K) (and all his paternal male relatives) is **included** as a potential donor of the major component. Using a published Y-STR DNA population database, this Y-STR profile has been observed in N of X total individuals within the Y-Chromosome Haplotype Reference Database (YHRD).*

*The Y-STR DNA profile from the major component **does not match** the Y-STR DNA profile from Known Sample Name (item K); he is excluded as a potential donor.*

7.2.5.7.14.4 **Y-STR Minor Component & Indistinguishable Mixture Profiles**

At this time, no inclusions will be made for Y-STR minor components or Y-STR indistinguishable mixtures. If a Y-

[35] Applicable wording may be used/modified for partial major components of major/minor mixture profiles, as necessary.

STR minor component or Y-STR indistinguishable mixture profile is obtained and a known reference sample cannot be excluded the following language should be use:

A Y-STR minor component/[partial] DNA mixture [of at least X individuals] was obtained [from Questioned Sample Description (item Q)].

No interpretative conclusions were made at this time due to there being no applicable statistic to convey the rarity of the profile applicable to any inclusionary statements.

– or –

OSBI policy does not currently allow for the interpretive conclusions of minor components of Y-STR mixtures/Y-STR indistinguishable mixtures; therefore, no comparison(s) or conclusions were made regarding the minor component/mixture obtained.

*The Y-STR DNA profile [from Questioned Sample Description (item Q)] appears to be a mixture. Known Sample Name (item K) is **excluded** as a potential contributor.*

7.2.5.7.15 **CODIS**

The following (or similar) wording will be added to each DNA report indicating whether any/which profiles were entered into the CODIS database:

7.2.5.7.15.1 **For cases with eligible profiles that are entered:** *The DNA profile(s) from item(s) (X…) was/were entered into the Combined DNA Index System (CODIS) database.*

7.2.5.7.15.2 **For cases where no profiles are identified that are eligible for entry:**

No DNA profiles were identified that were eligible for entry into the Combined DNA Index System (CODIS) database.

– or –

None of the DNA profiles obtained were eligible for entry into the Combined DNA Index System (CODIS) database.

7.2.5.7.15.3 **For cases with no results (no DNA profiles):** *None of the DNA results were eligible for entry into the Combined DNA Index System (CODIS) database.*

7.2.5.7.15.4 **For cases with Y-STR results only (with the possible exception of Missing Persons cases):** *The YSTR DNA profile(s) from item(s) (X…) was/were not entered into the Combined DNA Index System (CODIS) database at this time.*

7.2.5.7.16 **Disposition of Evidence**

7.2.5.7.16.1 **When items are returned:** *All evidence/list specific item(s) will be returned to the submitting/requesting agency for retention.*

7.2.5.7.16.2 **When items are retained, including DNA products:**
All evidence/list specific item(s) will be retained at the OSBI Forensic Science Center/Northeast Regional Laboratory.
– and/or –
All products of the DNA analysis process [pertaining to item(s) (X…)] will be retained at the OSBI Forensic Science Center/Northeast Regional Laboratory.

7.2.5.7.16.3 **When items are consumed:** *All evidence/list specific item(s) was/were consumed during testing.*

7.2.5.7.16.4 **When items are transferred between OSBI CSD Units:** *All evidence/list specific item(s) was/were transferred to the X Unit [to include associated derivative evidence removed from an item, if applicable].*

7.2.5.7.17 **Report Footnotes**

7.2.5.7.17.1 Each report will contain the following footnote: *This report contains the conclusions, opinions, and interpretations of the analyst whose signature appears on the report.*

7.2.5.7.17.2 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.*

7.2.5.7.17.3 A report footnote may be included clarifying epithelial and sperm fractions/portions, such as: *Items with "epithelial" and/or "sperm" fractions/portions were extracted using a differential DNA extraction method. This method aims to separate non-sperm cell DNA (epithelial fraction/portion) from potential sperm cell DNA (sperm fraction/portion). This terminology does not imply the presence or absence of spermatozoa and is not intended to indicate the sub-source of the DNA.*

7.2.5.7.17.4 If statistics are reported, the following footnotes will be included, as applicable:

7.2.5.7.17.4.1 **STR (including relationship/parentage):** *Statistics calculated using the OSBI STATS program "OSBI STATS, Version X," which calculates statistics for the Caucasian, African American, and Hispanic population groups using allele frequencies from the National Institute of Standards and Technology (NIST) 2017 revised Short Tandem Repeat DNA Internet Database (STRbase) at: http://www.cstl.nist.gov/div831/strbase/NISTpop.htm.*

7.2.5.7.17.4.1.1 **Statistical calculations reported that have dropout indicated in OSBI STATS due to low level/quality DNA (potential dropout):** *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 selecting an unrelated individual at random from the population who could also be a potential donor/contributor to the profile obtained.*

7.2.5.7.17.4.1.2 **Statistical calculations reported that have dropout indicated in OSBI STATS due to potential allele sharing only (not due to low level/quality DNA, for which the previous footnote would apply instead):** *The statistical calculation performed [for this item] accounts for potential allele sharing between the major and minor components and is a conservative estimate for the probability of selecting an unrelated individual at random from the population who could also be a potential donor/contributor to the profile obtained.*

7.2.5.7.17.4.2 **Y-STR:** *Statistics calculated using the Y-Chromosome Haplotype Reference Database (YHRD) at: http://www.yhrd.org/ (and version/release, if available).*

Attachment(s):
None

Reference(s):
A Guide for Determining What is Allowable in the Forensic Index at NDIS (i.e. NDIS Eligibility Chart)

Casework Standard Operating Procedures

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

SWGDAM Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories

OSBI Forensic Biology Units' Training Manual

CASE_8 RETENTION OF SAMPLES (↑ Table of Contents)

Quick link:
CWQM_7 (Facilities & Evidence Control)

8.1 Serology Samples

8.1.1 Blood Testing Work Products

8.1.1.1 **Hemastix®:** Not applicable.

8.1.1.2 **Takayama:** Slides generated while performing the Takayama test are to be disposed of after analysis of the sample is completed.

8.1.1.3 **RSID™-Blood:** Cuttings generated while performing the RSID™-Blood test are to be disposed of after analysis of the sample is completed.

8.1.2 Sexual Assault Testing Work Products

8.1.2.1 **AP Spot Test:** Cuttings generated while performing the AP Spot test are to be disposed of after analysis of the sample is completed.

8.1.2.2 **Seratec®:** Cuttings generated while performing the Seratec® test are to be disposed of after analysis of the sample is completed.

8.1.2.3 **Microscopic Identification of Spermatozoa:** Cuttings generated while performing the sperm cell search test are to be disposed of after analysis is completed. The slide prepared from these samples will be handled as outlined below:

8.1.2.3.1 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 (e.g. by placing a sticker with this indication on the outer packaging).

8.1.2.3.2 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

8.2.1 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), and QP 6.4 (Evidence Refrigerator and Freezer Maintenance).

8.2.2 Cuttings generated while performing the Y-screen assay are disposed of during analysis and crude Y-screen lysis extracts are to be disposed of after analysis of the sample is completed and will not be retained.

8.2.3 Cuttings, extracted DNA, and corresponding controls, at a minimum, shall be retained by the OSBI if the sample was consumed and extract remains, or if additional analysis may be necessary (e.g. Y-STR analysis).

8.2.3.1 If case circumstances warrant, the cuttings, extracts, and controls may be retained even if they do not fall under the above conditions.

8.2.3.2 Retention does not apply to cuttings taken for the Y-screen assay or resulting Y-screen crude lysis extracts.

8.2.3.3 Retention does not apply to hair root cuttings consumed during DNA analysis.

8.2.4 All retained DNA samples (e.g. cuttings, extracts, dilutions) should be stored frozen in a heat-sealed, clear plastic bag.

8.2.5 Amplified DNA products and genetic analyzer samples will 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**

8.3.1 The case notes should clearly describe what evidence is retained.

8.3.2 The case notes should clearly describe what cuttings, extracts, and dilutions were prepared and whether the samples were retained.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

FBI Quality Assurance Standards for DNA Databasing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

QC_1 CLEANING, DECONTAMINATION & STERILIZATION (↑ Table of Contents)

Quick link:
CWQM_9 (Analytical Procedures)

1.1 **Scope**
Quality control for cleaning and decontamination in the OSBI forensic biology units' laboratories.

1.2 **Reagents & Supplies**

1.2.1 1:10 bleach, alcohol and/or cleaner labeled as tuberculocidal

1.2.2 Detergent

1.3 **Equipment**

1.3.1 UV crosslinker

1.3.2 Autoclave

1.3.3 Dishwasher, if available

1.4 **Individual Steps of the Procedure**

1.4.1 **General Decontamination**

1.4.1.1 **Instruments**

1.4.1.1.1 Instruments used to process biological samples (e.g. forceps, scissors, etc.) shall be cleaned with an approximate 1:10 bleach solution or alcohol before and after each sample is handled.

1.4.1.1.2 Exposure to ultraviolet light for a minimum of 15 minutes in a UV crosslinker should be used to decontaminate articles that are small enough to fit into the unit and that will not be damaged by the ultraviolet light.

1.4.1.2 **Equipment**

1.4.1.2.1 Equipment shall be decontaminated with an approximate 1:10 bleach solution or alcohol (based on manufacturer recommendations and/or equipment type, e.g. use alcohol on stainless steel), periodically, as needed.

1.4.1.2.2 This shall be documented on the Decontamination Log (**QC_Decontamination_Log**) associated with the equipment.

1.4.1.3 **Floors**

1.4.1.3.1 Use an approximate 1:10 bleach solution for decontamination of floors, as necessary.

1.4.1.4 **Work Surfaces**

1.4.1.4.1 Work surfaces shall be cleaned with an approximate 1:10 bleach solution both before **and** after each use.

1.4.1.4.2 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.

1.4.1.5 **General Laboratory Glassware & Plasticware**

1.4.1.5.1 Glassware and plastic containers should be cleaned with detergent and completely rinsed with tap water, and then distilled water, by hand, immediately after use.

1.4.1.5.2 An automatic dishwasher may be used instead of handwashing.

1.4.1.5.2.1 If a dishwasher is used, glassware must be rinsed with distilled water either after washing or before use.

1.4.1.6 **Glassware & Plasticware Used to Handle Biohazards**

1.4.1.6.1 Clean and sterilize any glassware and/or plasticware used to handle biological samples following such a procedure:

1.4.1.6.1.1 Rinse item with an approximate 1:10 bleach solution or a cleaner labeled as tuberculocidal and then wash as above with detergent.

1.4.1.6.1.2 In addition to above, ultraviolet light exposure for ~15-30 minutes can be used for sterilization.

1.4.1.6.1.3 In addition to above, steam and/or heat sterilization in an autoclave can be used for items that will not melt.

1.4.1.7 **Solutions, Utensils & Tubes Necessary for DNA Analysis**

1.4.1.7.1 Sterilized by exposure to UV light for a minimum of 15 minutes in a UV crosslinker prior to use.

1.4.1.7.2 Only small aliquots of solutions should be used during analysis.

1.4.2 **Solutions & Supplies Requiring Sterilization**

1.4.2.1 **Autoclaving**

1.4.2.1.1 Autoclaving (sterilization) of solutions, if required, should be performed at the time of preparation following instructions within the operator's manual for use of the autoclave. Each respective reagent preparation procedure shall indicate if the solution requires sterilization by autoclaving.

1.4.2.1.2 Steam autoclaving of solutions should be performed at ~120°C for at least 20 minutes.

1.4.2.1.3 Use autoclave indicator tape on the containers.

1.4.2.1.4 After autoclaving is complete, check the indicator tape to make sure sterilization has taken place. If tape has not turned black, repeat procedure.

1.4.2.2 **Ultraviolet Crosslinker**

1.4.2.2.1 If crosslinking is required, items will be exposed to UV light (short wave: 254nm) for a minimum of 15 minutes following instructions within the operator's manual for use of the crosslinker.

1.4.2.2.2 The crosslinker should be equipped with short wave (254 nm) tubes in order to ensure decontamination.

1.4.3 **Decontamination Following a Contamination Event**

1.4.3.1 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:

1.4.3.1.1 Discard facemask(s) and obtain a new facemask.

1.4.3.1.2 Remove laboratory coat(s) from the laboratory and have it/them cleaned or obtain a new disposable lab coat.

1.4.3.1.3 Dispose of aliquots of reagents, such as extraction reagents, TE^{-4}, and any kits/reagents that have been tested and shown to be contaminated.

1.4.3.1.4 Decontaminate all supplies (e.g. pipettors, racks, decappers, etc.) and equipment (e.g. centrifuges, hoods, etc.) used throughout the analysis process with an approximate 1:10 bleach solution or alcohol (based on manufacturer recommendations and/or equipment type, e.g. use alcohol on stainless steel).

1.4.3.1.5 Decontaminate all work areas, including common areas, potentially used throughout the analysis process with an approximate 1:10 bleach solution.

1.4.3.1.6 UV crosslink all small supplies and reagents associated with the analysis process.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Decontamination_Log**

Reference(s):
Systec DX/DE Series Operating Manual (autoclave user guide)

Spectroline Microprocessor-Controlled UV Crosslinkers Operator's Manual

SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

QC_2 CROSSLINKERS (↑ Table of Contents)

2.1 **Scope**
Quality control for crosslinker to ensure optimal operation.

2.2 **Reagents & Supplies**

2.2.1 Crosslinker bulb (short wave, 254 nm)

2.3 **Equipment**

2.3.1 Crosslinker

2.4 **Individual Steps of the Procedure**

2.4.1 **Performance Check**

2.4.1.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check for each crosslinker.

2.4.1.2 The procedure listed in the operator's manual shall be used to check the intensity of the bulbs.

2.4.1.3 If the performance check is not completed within the anniversary month of the last performance check, the crosslinker shall be removed from service until a performance check is successfully completed.

2.4.1.4 Document the results on the general Equipment Maintenance Log (**QC_Equip_Maint_Log**).

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Equip_Maint_Log**

Reference(s):
Spectroline Microprocessor-Controlled UV Crosslinkers Operator's Manual

QC_3 NIST CERTIFIED EQUIPMENT (↑ Table of Contents)

3.1 **Scope**
Quality control for NIST certified equipment to ensure optimal operation.

3.2 **Equipment**

3.2.1 Any NIST certified equipment, such as weights, timers, tachometers, temperature verification systems, thermometers, etc.

3.3 **Individual Steps of the Procedure**

3.3.1 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.

3.3.2 Contact a company/business that is an authorized NIST certifying body and meets the accreditation requirements for an external service provider, such as Probata Corporation at (405) 607-4813, or the NIST Calibration Program at (301) 975-2092 or calibrations@nist.gov to arrange for the calibration.

3.3.3 Once an IPR is approved, deliver to the agency directed by the NIST representative for the calibration.

3.3.4 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.

3.3.5 Calibration and maintenance records for NIST certified equipment will be maintained accordingly.

3.4 **Notes**

3.4.1 It may be more cost effective to replace NIST certified timers at the end of their current certificate rather than to send for recalibration.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

International Standard ISO/IEC 17025:2017 General Requirements for the Competence of Testing and Calibration Laboratories

ANAB ISO/IEC 17025:2017 – Forensic Science Testing and Calibration Laboratories Accreditation Requirements (AR 3125)

QC_4 REFRIGERATORS & FREEZERS (↑ Table of Contents)

4.1 **Scope**
Quality control for refrigerators and freezers to ensure optimum operation.

4.2 **Equipment**

4.2.1 Refrigerator/freezer (with thermometer to measure temperature)

4.3 **Individual Steps of the Procedure**

4.3.1 At least once a week, refrigerator and freezer temperatures shall be checked and recorded on the Temperature Log (**QC_Temp_Log**), or, alternatively, temperatures may be recorded in the designated Excel workbook (**QC_Compiled_Temp_Logs_&_Safety_QC**). All records will be maintained in the appropriate location on the network.

4.3.1.1 The weekly temperature should not be recorded when the unit is in defrost mode.

4.3.1.2 The individual 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 unsatisfactory.

4.3.1.3 If the weekly check results in a temperature outside of the expected temperature range, the individual performing the temperature check shall check again in 15 to 30 minutes or make an adjustment to the temperature setting and repeat the check in 30 minutes.

4.3.1.4 If the temperature remains outside the acceptable range, the individual performing the check shall notify the unit supervisor immediately and take the action described below for unsatisfactory unit performance.

4.3.1.5 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.

4.4 **Interpretation of Results (Refrigerator/Freezer Performance)**

4.4.1 For unsatisfactory unit performance (i.e. temperature outside of expected):

4.4.1.1 The unit shall be inspected and the cause of any variance determined and corrected.

4.4.1.2 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 or the unit replaced.

4.4.1.2.1 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.

4.4.1.2.2 The date the unit is placed out of service shall be recorded on the Temperature Log (**QC_Temp_Log**) or in the designated Excel workbook (**QC_Compiled_Temp_Logs_&_Safety_QC**) and maintained in the appropriate location on the network.

4.4.1.2.3 The unit supervisor or designee shall arrange to have the unit repaired or replaced.

4.4.2 If a unit sets off an alarm, the alarm monitoring company, as per contract, should contact the designated contact person for that laboratory in a timely manner. 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.

4.5 **Notes**

4.5.1 Standard refrigerators should generally maintain a temperature range of approximately 1˚C to 10˚C. Standard freezers should generally maintain a temperature range of approximately -30˚C to 0˚C. Specific protocols that specify a temperature range shall supersede the aforementioned ranges.

4.5.2 Refrigerator/freezer units with frost-free functions have short duration defrost cycles. 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, and the alarm monitoring pad should be set to take the variance into account.

4.5.3 Each thermometer should be placed in an easily visible location. The bulb, sensor, or probe portion should be free from contact with any other item or material.

4.5.4 Unless otherwise specified in superseding protocols, thermometers do not need to be NIST traceable or require any calibration.

4.5.5 The contents of each refrigerator/freezer unit should be arranged and reasonably limited so that the proper circulation of air within the unit is maintained.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Temp_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Compiled_Temp_Logs_&_Safety_QC**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

QC_5 BALANCE (↑ Table of Contents)

5.1 **Scope**
Quality control for balance to ensure optimum operation.

5.2 **Equipment**

5.2.1 Balance

5.3 **Individual Steps of the Procedure**

5.3.1 **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 for cleaning.

5.3.2 **Performance Check**

5.3.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on each balance. The performance check will be performed by an external vendor in accordance with OSBI CSD QM 6.5 (Metrological Traceability) and QP 24 (Calibration and Handling of Equipment).

5.3.2.2 If the performance check is not completed within the anniversary month of the last performance check, the balance shall be removed from service until a performance check is successfully completed.

5.3.3 **Required Documentation**

5.3.3.1 Archive the certification from the external vendor in the appropriate network folder as documentation of the successful completion of the performance check.

5.3.3.2 If no certificate is available, the performance check will be documented using the general Equipment Maintenance Log (**QC_Equip_Maint_Log**) and maintained in the appropriate location on the network.

5.4 **Interpretation of Results (Balance Performance)**

5.4.1 If any balance fails to perform within the accepted range, troubleshooting procedures provided in that individual model's instructions and operator's manual should be followed.

5.4.2 If problems persist that cannot be corrected in the laboratory, the balance shall be serviced by an authorized service center.

5.4.3 Certification from the external vendor shall serve as the successful completion of the performance check.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Equip_Maint_Log**

Reference(s):
Mettler Toledo Excellence Analytical Balances XSE Models Operating Instructions

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

QC_6 PIPETTORS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

6.1 **Scope**
Quality control for pipettors (mechanical pipettes) to ensure optimal operation.

6.2 **Reagents & Supplies**

6.2.1 Weighing receptacle (e.g. water trap or weigh boat)

6.2.2 Deionized water

6.3 **Equipment**

6.3.1 Mechanical pipet

6.3.2 Balance (calibrated)

6.3.3 Calibri software, if available

6.4 **Individual Steps of the Procedure**

6.4.1 **Performance Check**

6.4.1.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on all pipettors. The annual performance check may be completed in-house or by an external vendor.

6.4.1.2 If the performance check is not completed within the anniversary month of the last performance check, the pipettor shall be removed from service until a performance check is successfully completed.

6.4.2 **Pipettor Type Requirements**

6.4.2.1 Adjustable pipettors shall be checked at the minimum and maximum values, with the exception of the 2µL and 10µL pipettors, which will be checked using 1µL for the minimum value. For Rainin pipettors, the range for P-2 is 0.1 to 2µL, P-10 is 0.5 to 10µL, P100 is 10 to 100µL, P-200 is 50 to 200µL and P-1000 is 100 to 1000µL.

6.4.2.2 Repeat pipettors shall be checked at the minimum and maximum values based on the dispensing volume range associated with the attached tip, and they shall be labeled with the approved volume range.

6.4.2.3 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.

6.4.3 **Performing an In-House Performance Check**

6.4.3.1 Using a calibrated balance, place a weighing receptacle on the balance and tare.

6.4.3.2 Deliver the desired volume of water to the weighing receptacle and record the registered weight. This may be accomplished by using the Calibri software recording the measured volume.

6.4.3.3 If taking measurements by hand, calculate % error for each measurement using the formula:

(Delivered Weight – Expected Weight) / Expected Weight x 100%

6.4.4 **Required Documentation**

6.4.4.1 Document results of the in-house performance check for each pipettor using the Pipettor Performance Check Log (**QC_Pipet_PC_Log**) and/or the Pipettor Performance Check Reproducibility Log (**QC_Pipet_PC_Reproducibility_Log**) and maintained on the network or archive the "Calibration Report" from the Calibri software in the appropriate QC folder on the network.

6.4.4.2 Certification from the external vendor shall serve as the successful completion of the performance check, including for any pipet sent to an external vendor for adjustment/maintenance. 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.

6.4.4.2.1 Calibration labs fulfilling the requirements of ISO 17025 are considered competent. Copies of ISO 17025 certificates shall be maintained with the calibration/test report as sufficient evidence of traceability of the calibration data reported. A review of the certificate(s) by the technical manager and/or Lead Analyst (or designee) shall serve as documentation of this check of compliance regarding function and calibration.

6.5 **Interpretation of Results (Pipettor Performance)**

6.5.1 Determine whether the pipettor passed or failed the performance check. The tolerance for pipettor volumes greater than or equal to 10µL is 3%. The tolerance range for volumes less than 10µL is 10%.

6.5.2 If the percent error is greater than the 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_Pipet_PC_Reproducibility_Log**). If the percent error is within the established tolerance, the pipettor is accepted as meeting criteria.

6.5.3 If the pipettor fails after five tries, adjustment/maintenance is necessary. This can be done in-house or at an authorized service center (external vendor).

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Pipet_PC_Log**

OSBI Forensic Biology Units' Policy Manual **QC_Pipet_PC_Reproducibility_Log**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

OSBI Forensic Biology Units' Training Manual

QC_7 THERMOMETERS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

7.1 **Scope**
Quality control for thermometers to ensure optimum operation.

7.2 **Equipment**

7.2.1 Thermometer

7.2.2 Water bath

7.2.3 NIST certified thermometer

7.3 **Individual Steps of the Procedure**

7.3.1 **Performance Check**

7.3.1.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on each NIST traceable thermometer used for conducting performance checks. The annual performance check can be accomplished either through certification by an external vendor or through verification in-house.

7.3.1.2 If the performance check is not completed within the anniversary month of the last performance check, the thermometer shall be removed from service until a performance check is successfully completed.

7.3.1.3 Perform an in-house performance check as follows:

7.3.1.3.1 Set a water bath to the desired temperature.

7.3.1.3.2 Allow water to heat for at least 30 minutes.

7.3.1.3.3 Place bulb of each thermometer to be checked into the water bath along with the NIST certified thermometer.

7.3.1.3.4 Wait several minutes and record the temperature on the Thermometer Performance Check Log (**QC_Thermometer_PC_Log**).

7.3.1.3.5 Wait 15 minutes and record the temperature again.

7.3.1.3.6 Alternatively, the calibrating digital thermometer can be placed into the same equipment as the thermometer, be allowed to equilibrate, and the reading documented.

7.3.2 **Required Documentation**

7.3.2.1 Archive the certification from the external vendor in the appropriate network folder as documentation of the successful completion of the performance check. If no certificate is available, the performance check will be documented on the Thermometer Performance Check Log (**QC_Thermometer_PC_Log**) and maintained in the appropriate location on the network.

7.3.2.2 In-house performance checks will be documented on the Thermometer Performance Check Log (**QC_Thermometer_PC_Log**) maintained in the appropriate location on the network.

7.4 **Interpretation of Results (Thermometer Performance)**

7.4.1 If thermometer readings equal the NIST calibrated reading, no action is needed.

7.4.2 If the NIST thermometer measurement is within ±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.

7.4.3 If the NIST thermometer measurement is more than ±1 degree of the thermometer being tested, the thermometer shall be replaced or taken out of service.

7.4.4 Certification from the external vendor shall serve as the successful completion of the performance check.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Thermometer_PC_Log**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

QC_8 TEMPERATURE VERIFICATION SYSTEM (TVS) (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

8.1 **Scope**
Quality control for temperature verification system (TVS) (or temperature verification kit [TVK]) to ensure optimal operation.

8.2 **Equipment**

8.2.1 Temperature verification system (TVS)

8.3 **Individual Steps of the Procedure**

8.3.1 **Performance Check**

8.3.1.1 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 thermomixers and thermal cyclers (when performed in-house).

8.3.1.2 If the performance check is not completed within the anniversary month of the last performance check, the TVS shall be removed from service until a performance check is successfully completed.

8.3.2 **Required Documentation**

8.3.2.1 Archive the certification from the external vendor in the appropriate network folder as documentation of the successful completion of the performance check.

8.4 **Interpretation of Results (TVS Performance)**

8.4.1 Certification from the external vendor shall serve as the successful completion of the performance check.

Attachment(s):
None

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

FBI Quality Assurance Standards for Forensic DNA Testing Laboratories

SWGDAM Guidance Document for the FBI Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories

QC_9 THERMOMIXERS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

9.1 **Scope**
Quality control for thermomixers to ensure optimum operation.

9.2 **Reagents & Supplies**

9.2.1 Neutral cleaning agent

9.3 **Equipment**

9.3.1 Eppendorf ThermoMixer® C

9.3.2 Temperature Verification System (TVS) (certified)

9.3.3 Stopwatch or other certified timer

9.4 **Individual Steps of the Procedure**

9.4.1 **Cleaning**

9.4.1.1 Use neutral cleaning agents to clean the ThermoMixer® housing and thermoblocks, as needed.

9.4.1.2 Ensure that all parts are dried thoroughly before use.

9.4.2 **Performance Check**

9.4.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check for each ThermoMixer® consisting of both a temperature and timer verification.

9.4.2.2 If the performance check is not completed within the anniversary month of the last performance check, the ThermoMixer® shall be removed from service until a performance check is successfully completed.

9.4.2.3 **Temperature Verification**

9.4.2.3.1 Remove the 0.02mL well cover located in the middle of the smart block (if present).

9.4.2.3.2 Verify the appropriate temperatures used in policy (see DNA_1, DNA_2 and DNA_3) with a Temperature Verification System (TVS).

9.4.2.3.3 Document the results on the ThermoMixer® Performance Check Log (**QC_Thermomixer_PC_Log**).

9.4.2.4 **Timer Verification**

9.4.2.4.1 Measure the timer against a stopwatch or other calibrated timer set for the same time interval.

9.4.2.4.2 Document the results on the ThermoMixer® Performance Check Log (**QC_Thermomixer_PC_Log**).

9.5 **Interpretation of Results (ThermoMixer® Performance)**

9.5.1 **Temperature**

9.5.1.1 If the temperature is more than 1.0°C above or below the desired temperature, the test is to be run two more times.

9.5.1.2 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.

9.5.2 **Timer**

9.5.2.1 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):
OSBI Forensic Biology Units' Policy Manual **QC_Thermomixer_PC_Log**

Reference(s):
Eppendorf ThermoMixer® C Operating Manual

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QC_10 PROFLEX™ PCR SYSTEMS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

10.1 **Scope**

Quality control for Applied Biosystems™ ProFlex™ PCR Systems to ensure optimum thermal cycler operation.

10.2 **Equipment**

10.2.1 Applied Biosystems™ ProFlex™ PCR System

10.2.2 Temperature Verification Kit (TVK) (certified)

10.3 **Individual Steps of the Procedure**

10.3.1 **Maintenance Schedule**

10.3.1.1 **Monthly**

10.3.1.1.1 Perform the Self-Verification Test (SVT):

10.3.1.1.1.1 In the Home Screen, select **Settings**, then **Maintenance & Services**, then **Self Verification Test**.

10.3.1.1.1.2 In the Self Verification Test screen, select **Start Test** (test take about 10 minutes and the results are displayed in the form of a report).

10.3.1.1.1.3 Save the test report to the USB drive by selecting **Export**.

10.3.1.1.1.4 Record completion of test and results.

10.3.1.1.2 Clean sample block/wells.

10.3.1.1.3 Clean heated cover.

10.3.1.2 **Quarterly**

10.3.1.2.1 Check the software settings to ensure they remain aligned with policy specifications

10.3.1.3 **Annually**

10.3.1.3.1 Performance check (see below).

10.3.1.4 **As Needed**

10.3.1.4.1 Replace fuses.

10.3.1.4.2 Upgrade system firmware (in consultation with technical manager and/or Lead Analyst).

10.3.1.4.3 Decontaminate sample block/wells.

10.3.1.4.4 Decontaminate heated cover.

10.3.2 **Annual Performance Check (Planned/Preventative Maintenance)**

10.3.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on each ProFlex™ PCR System. The annual performance check can be achieved by the planned/preventative maintenance (PM) of the instrument when performed by a qualified service technician from an external vendor (e.g. manufacturer), or, if a PM is not performed, a performance check will be completed using a Temperature Verification Kit (TVK).

10.3.2.2 If the performance check is not completed within the anniversary month of the last performance check, the ProFlex™ PCR System shall be removed from service until a performance check is successfully completed.

10.3.3 **Performance Check Following Repair, Service or Calibration**

10.3.3.1 Each ProFlex™ PCR System shall undergo a performance check after any repair, service or calibration and before use in casework. The performance check will be completed in-house using either the Self-Verification Test (SVT) and/or the Temperature Verification Kit (TVK), as indicated by the technical manager and/or Lead Analyst; or alternatively, certification from an external vendor may also serve as the successful completion of the performance check.

10.3.4 **Required Documentation**

10.3.4.1 Record all maintenance tasks and performance checks performed on the on the ProFlex™ PCR System Maintenance Log (**QC_ProFlex_Maint_Log**) and/or ProFlex™ PCR System Performance Check Log (**QC_ProFlex_PC_Log**) and maintain in the appropriate network folder.

10.3.4.2 Record completion of the Self-Verification Test (SVT) on the ProFlex™ PCR System Maintenance Log (**QC_ProFlex_Maint_Log**). Results must either be documented on the ProFlex™ PCR System Performance Check Log (**QC_ProFlex_PC_Log**), or the instrument logs may be exported and stored digitally in the appropriate quality record folder. If a SVT is performed by a qualified service technician, the instrument logs and service call forms will be stored in the appropriate quality record folder.

10.3.4.3 Archive the certification from the external vendor, such as field service reports, in the appropriate network folder as documentation of the successful completion of the performance check.

10.4 **Interpretation of Results (ProFlex™ PCR System Performance)**

10.4.1 If a ProFlex™ PCR System fails to perform correctly, the self-verification test may be repeated and the technical manager, Lead Analyst, and supervisor will be notified.

10.4.2 If continued attempts fail, the instrument shall be removed from service and a qualified service technician shall be contacted.

10.4.3 Certification from the external vendor shall serve as the successful completion of the performance check.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_ProFlex_Maint_Log**

OSBI Forensic Biology Units' Policy Manual **QC_ProFlex_PC_Log**

Reference(s):
Life Technologies™ ProFlex™ PCR System User Guide

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QC_11 3500 SERIES GENETIC ANALYZERS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

11.1 Scope

Quality control for the 3500 Series Genetic Analyzer. This protocol summarizes common tasks required to properly maintain and calibrate the 3500 Series Genetic Analyzer under normal conditions. Other service work must be referred to a qualified service technician.

11.2 Reagents & Supplies

11.2.1 Capillary array

11.2.2 Anode Buffer Container (ABC)

11.2.3 Cathode Buffer Container (CBC)

11.2.4 Performance Optimized Polymer 4 (POP-4™)

11.2.5 Conditioning Reagent

11.2.6 HID Install Standard

11.2.7 Hi-Di™ Formamide

11.2.8 GeneScan™ 600 LIZ™ Size Standard v2.0 (GS600 LIZ™ v2.0)

11.2.9 Applied Biosystems™ DS-36 (Dye Set J6) Matrix Standard Kit

11.2.10 Deionized water

11.2.11 Syringe

11.2.12 Kimwipe or equivalent lint-free cloth

11.2.13 96-well optical reaction plate

11.2.14 96-well septa mat

11.2.15 Plate base and retainer

11.3 Equipment

11.3.1 Applied Biosystems™ 3500 Series Genetic Analyzer

11.3.2 Mechanical pipettors

11.3.3 Vortex

11.3.4 MiniSpin

11.3.5 Centrifuge

11.3.6 Available equipment for performing heat denature/snap cool (e.g. thermal cycler/freezer, ice or cold block)

11.4 Individual Steps of the Procedure

11.4.1 Maintenance Schedule

The following outlines the schedule for the specific maintenance tasks needed to ensure optimal performance of the 3500 Genetic Analyzer and computer. Tasks can be performed less often if the instrument is not in use, as long as the tasks are performed before the next use.

11.4.1.1 **Daily** (or before each run when instrument is not used daily)

11.4.1.1.1 Check Dashboard.

11.4.1.1.2 Visually inspect the fluid level in the buffer containers.

11.4.1.2 **Weekly** (every 7 days when instrument is in use)

11.4.1.2.1 Check storage condition of used array to ensure array tip is covered in the reservoir.

11.4.1.2.2 Record number of runs.

11.4.1.2.3 Run Wash Pump and Channels Wizard (i.e. water wash).

11.4.1.3 **Bi-Weekly**

11.4.1.3.1 Replace anode and cathode buffers.

11.4.1.3.2 Replace septa on reservoirs.

11.4.1.3.3 Replace POP-4™ pouch.

11.4.1.4 **Monthly**

11.4.1.4.1 Flush pump trap.

11.4.1.4.2 Empty condensation container and water trap waste container.

11.4.1.4.3 Examine instrument for any residue and, if present, wipe with deionized water and a lint-free cloth.

11.4.1.5 **Quarterly**

11.4.1.5.1 Check the software settings to ensure they remain aligned with policy specifications

11.4.1.6 **Annually**

11.4.1.6.1 Performance check (see below).

11.4.1.7 **As Needed**

11.4.1.7.1 Restart the computer.

11.4.1.7.2 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.

11.4.1.7.3 Perform 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 the detector door is opened or the detection cell is moved; or when the instrument is moved.

11.4.1.7.4 Perform spectral calibration after replacing a capillary array; if there is a change to the dye set or polymer type; when a service technician performs an optical service procedure, such as realigning or replacing the laser, CCD cameras or mirrors; or when an increase of spectral separation (pull-up/pull-down peaks) is observed in the raw or analyzed data.

11.4.1.7.4.1 Place a copy of the Spectral Report in the QC folder on the instrument computer desktop.

11.4.1.7.5 Defragment the computer hard drive. The fragmentation of files decreases the performance of the Data Collection Software and the computer operating system; programs take a longer time to access files by performing multiple search operations of the fragments.

11.4.1.7.5.1 In the Windows desktop, select **Start** > **Programs** > **Accessories** > **System Tools** > **Disk Defragmenter** and follow the prompts. **(**Note: You can click **Analyze** to see if you should defragment or not.)

11.4.1.7.5.1.1 Do not manually delete spectral plate records or spectral information from the database. Doing so will permanently delete spectral information.

11.4.1.7.5.2 Once complete, a dialog box will appear and allow you to view a report or close.

11.4.1.7.6 Archive the Library to the appropriate location on the network.

11.4.1.7.7 Run the HID Install Standard.

11.4.2 **Annual Performance Check (Planned/Preventative Maintenance)**

11.4.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on each 3500 Series Genetic Analyzer. The performance check performed after the annual planned/preventative maintenance (PM) from an external vendor (e.g. manufacturer) will serve as the annual performance check.

11.4.2.2 The annual PM will be performed by a qualified service technician.

11.4.2.3 Following the completion of the annual PM, a performance check will be performed for each 3500 Series Genetic Analyzer to verify the instrument conforms to analysis sizing precision, sizing range, and peak height specifications.

11.4.2.3.1 The performance check will consist of the evaluation of the first successful positive control, negative control, and ladder ran on the instrument. This performance check is required prior to the acceptance of data. These controls may be run concurrently with casework samples, but if the controls fail, no data from the plate will be accepted.

11.4.2.3.2 If instrument sensitivity concerns are present, the technical manager will be notified and a dilution series shall be analyzed at the instruction of the technical manager and/or Lead Analyst.

11.4.2.4 If the PM and performance check are not completed within the anniversary month of the last performance check, the 3500 Series Genetic Analyzer shall be removed from service until they have been successfully completed.

11.4.3 **Performance Check Following Installation of Capillary Array or Repair, Service or Calibration**

11.4.3.1 A performance check will be performed for each 3500 Series Genetic Analyzer to verify the instrument conforms to analysis sizing precision, sizing range, and peak height specifications after the replacement of the capillary array (including reinstallation of one that has been stored) or following any repair, service or calibration, including when alterations to the optics of the instrument (i.e. laser or other optical components) have been performed.

11.4.3.2 The performance check will consist of the evaluation of the first successful positive control, negative control, and ladder ran on the instrument and is required prior to the acceptance of data. This performance check is required prior to the acceptance of data. These controls may be run concurrently with casework samples, but if the controls fail, no data from the plate will be accepted.

11.4.3.3 If instrument sensitivity concerns are present, the technical manager will be notified and a dilution series shall be analyzed at the instruction of the technical manager and/or Lead Analyst.

11.4.4 **Required Documentation**

11.4.4.1 Place a copy of the Spectral Report in the QC folder on the instrument computer desktop.

11.4.4.2 Record all maintenance tasks performed on the 3500 Genetic Analyzer Maintenance and Performance Check Log (**QC_3500_Maint_&_PC_Log**) on the network.

11.4.4.3 Record all performance checks performed on the 3500 Genetic Analyzer Maintenance and Performance Check Log (**QC_3500_Maint_&_PC_Log**) on the network. In addition, document the results of the performance check by saving them to the appropriate network folder, including the electropherograms of the controls.

11.4.4.4 Archive the certification from the external vendor, such as field service reports, in the appropriate network folder.

11.5 **Interpretation of Results (3500 Series Genetic Analyzer Performance)**

11.5.1 If a 3500 Series 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.

11.5.2 If the 3500 Series Genetic Analyzer continues to yield unsatisfactory performance, the instrument shall be removed from service and a qualified service technician shall be contacted.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_3500_Maint_&_PC_Log**

Reference(s):
Applied Biosystems™ 3500/3500xL Genetic Analyzer User Guide

Applied Biosystems™ 3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 4.0 User Guide

Applied Biosystems™ Multi-Capillary DS-36 Matrix Standard (Dye Set J6) Product Insert

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QC_12 AUTOMATE *EXPRESS*™ FORENSIC DNA EXTRACTION (ROBOTIC) SYSTEMS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

12.1 **Scope**

Quality control for AutoMate *Express*™ Forensic DNA Extraction System.

12.2 **Reagents & Supplies**

12.2.1 Alcohol

12.2.2 Mild detergent

12.2.3 Silicon grease

12.2.4 D-rings

12.3 **Equipment**

12.3.1 AutoMate *Express*™ Forensic DNA Extraction System

12.4 **Individual Steps of the Procedure**

12.4.1 **Maintenance Schedule**

The maintenance tasks necessary to ensure optimal performance of the AutoMate *Express*™ Forensic DNA Extraction System are detailed below and shall be performed as per the instrument user guide.

12.4.1.1 **Daily** (or before each run when instrument is not used daily)

12.4.1.1.1 Clean piercing units with alcohol.

12.4.1.2 **Biweekly**

12.4.1.2.1 Apply silicon grease to the D-rings of the nozzle.

12.4.1.2.2 Clean the cartridge rack, tip and tube rack, and magnets with a mild detergent allowing the parts to dry before use.

12.4.1.3 **Monthly**

12.4.1.3.1 Perform axis and temperature tests.

12.4.1.4 **Annually**

12.4.1.4.1 Replace the D-rings.

12.4.1.4.2 Performance check (see below).

12.4.1.5 **As Needed**

12.4.1.5.1 Clean the cartridge rack, tip and tube rack, and magnets with a mild detergent allowing the parts to dry before use.

12.4.1.5.2 While the instrument is off, remove and clean bottom tray with mild detergent and dry before use.

12.4.1.5.3 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.

12.4.2 **Annual Performance Check (Planned/Preventative Maintenance)**

12.4.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check on each AutoMate *Express*™ Forensic DNA Extraction System. The annual performance check can be achieved by the planned/preventative maintenance (PM) of the instrument when performed by a qualified service technician from an external vendor (e.g. manufacturer). If a PM is not performed, QC of a new PrepFiler *Express*™ or PrepFiler *Express* BTA™ Forensic DNA Extraction Kit using two NIST traceable samples shall serve as the annual performance check.

12.4.2.2 If the performance check is not completed within the anniversary month of the last performance check, the instrument shall be removed from service until a performance check is successfully completed.

12.4.3 **Performance Check Following Repair, Service or Calibration**

12.4.3.1 Each AutoMate *Express*™ Forensic DNA Extraction System shall undergo a performance check after any repair, service or calibration and before use in casework. Certification from an external vendor may serve as the successful completion of the performance check, or alternatively, QC of a new PrepFiler *Express*™ or PrepFiler *Express* BTA™ Forensic DNA Extraction Kit using two NIST traceable samples shall serve as the performance check.

12.4.4 **Required Documentation**

12.4.4.1 Record all maintenance tasks and performance checks performed on the AutoMate *Express*™ Maintenance Log (**QC_AutoMate_Maint_Log**).

12.4.4.2 Archive the certification from the external vendor, such as field service reports, in the appropriate network folder as documentation of the successful completion of the performance check.

12.5 **Interpretation of Results (AutoMate *Express*™ Forensic DNA Extraction System Performance)**

12.5.1 If a PM is not performed, QC of a new PrepFiler *Express*™ or PrepFiler *Express* BTA™ Forensic DNA Extraction Kit using two NIST traceable samples shall serve as the successful completion of the performance check as long as the samples achieve the expected quantitation results.

12.5.2 Certification from the external vendor shall serve as the successful completion of the performance check.

12.6 **Notes**

12.6.1 Do not use acid or bases (bleach) to clean the instrument.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_AutoMate_Maint_Log**

Reference(s):
Life Technologies™ AutoMate *Express*™ Instrument User Guide

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

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QC_13 QUANTSTUDIO™ 5 REAL-TIME PCR SYSTEMS (↑ Table of Contents)

Quick link:
CWQM_10 (Equipment)

13.1 **Scope**
Quality control for Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Systems.

13.2 **Equipment**

13.2.1 Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System

13.3 **Individual Steps of the Procedure**

13.3.1 **Maintenance Schedule**

13.3.1.1 **Monthly**

13.3.1.1.1 Perform a background calibration.

13.3.1.1.2 Perform the Self-Verification Test (SVT).

13.3.1.2 **Quarterly**

13.3.1.2.1 Check the software settings to ensure they remain aligned with policy specifications

13.3.1.3 **Annually**

13.3.1.3.1 Performance check (see below).

13.3.1.4 **As Needed**

13.3.1.4.1 Restart the computer.

13.3.1.4.2 Check disk space.

13.3.1.4.3 Run disk cleanup and defragment the computer hard drive.

13.3.1.4.4 Decontaminate the sample block.

13.3.1.4.5 Replace the instrument fuses.

13.3.1.4.6 Perform a background calibration.

13.3.1.4.7 Run a standard curve dilution series (checks all dye filters) or perform a RNase P instrument verification (only checks FAM dye filter) to confirm instrument performance.

13.3.2 **Annual Performance Check (Planned/Preventative Maintenance)**

13.3.2.1 An annual performance check will be performed on each QuantStudio™ 5 Real-Time PCR System. The annual planned/preventative maintenance (PM) performed

by an external vendor (e.g. manufacturer) will serve as the annual performance check.

13.3.2.2 The annual PM will be performed by a qualified service technician.

13.3.2.3 If the performance check is not completed within the anniversary month of the last performance check, the QuantStudio™ 5 Real-Time PCR System shall be removed from service until a performance check is successfully completed.

13.3.2.4 Following completion of the annual PM, the threshold values in the C_T setting tab will be verified as accurate (T.IPC Threshold = 0.1, T.Large Auto, T.Small Auto, T.Y = 0.2).

13.3.3 **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, and the threshold values in the C_T setting tab will be verified as accurate (T.IPC Threshold = 0.1, T.Large Auto, T.Small Auto, T.Y = 0.2).

13.3.4 **Required Documentation**

13.3.4.1 Record all maintenance tasks and performance checks performed on the QuantStudio™ 5 Real-Time PCR System Maintenance Log (**QC_QS5_Maint_Log**) on the network.

13.3.4.2 Archive the certification from the external vendor, such as field service reports, in the appropriate network folder as documentation of the successful completion of the performance check.

13.4 **Interpretation of Results (QuantStudio™ 5 Real-Time PCR System Performance)**

13.4.1 Certification from the external vendor shall serve as the successful completion of the performance check.

13.5 **Notes**

13.5.1 Signals that exceed the limit of normal fluorescence may indicate fluorescent contaminants on the calibration plate or the sample block. Common contaminants include ink residue from permanent pens/markers, powder from disposable gloves, and dust. Follow the steps outlined in the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (for Human Identification) User Guide to identify and resolve a possible contaminant.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_QS5_Maint_Log**

Reference(s):

Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (for Human Identification) User Guide

Applied Biosystems™ HID Real-Time PCR Analysis Software Version 1.3 User Guide

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OSBI Forensic Biology Units' Training Manual

QC_14 CENTRIFUGES (↑ Table of Contents)

14.1 **Scope**

Quality control for centrifuges.

14.2 **Equipment**

14.2.1 Centrifuge

14.2.2 Tachometer (certified)

14.2.3 Stopwatch or other certified timer

14.3 **Individual Steps of the Procedure**

14.3.1 **Cleaning**

14.3.1.1 Use neutral cleaning agents to clean the centrifuge housing, rotor chamber, and rotor accessories.

14.3.1.2 Ensure that all parts are dried thoroughly by hand before use.

14.3.2 **Performance Check**

14.3.2.1 At a minimum, the OSBI forensic biology units shall perform an annual performance check for each centrifuge consisting of both a RPM check and timer check.

14.3.2.2 If the performance check is not completed within the anniversary month of the last performance check, the centrifuge shall be removed from service until a performance check is successfully completed.

14.3.2.3 Performance checks are required for only those centrifuges used where the RPM and time measurements are required in casework; performance checks are not required for centrifuges designated as “QUICK SPIN ONLY.”

14.3.2.4 Perform the performance check as follows:

14.3.2.4.1 **RPM Check**

14.3.2.4.1.1 The speed (RPM) as measured by a tachometer using a strobe light.

14.3.2.4.1.2 Ensure that there is a reflective surface on the rotor of the centrifuge to be checked that will register when the tachometer is activated.

14.3.2.4.1.3 Turn on the centrifuge and allow the centrifuge to come to maximum rotation.

14.3.2.4.1.4 Follow the operator’s manual for the tachometer to begin the check of the centrifuge.

14.3.2.4.1.5 Document RPM check results on the Centrifuge Performance Check Log (**QC_Centrifuge_PC_Log**).

14.3.2.4.2 **Timer Check**

14.3.2.4.2.1 Measure the timer against a stopwatch or other calibrated timer set for the same time interval of five minutes.

14.3.2.4.2.2 Document timer check results on the Centrifuge Performance Check Log (**QC_Centrifuge_PC_Log**).

14.4 **Interpretation of Results (Centrifuge Performance)**

14.4.1 **RPM**

14.4.1.1 RPM must fall within the desired range of ± 10% of maximum setting.

14.4.1.2 Moderate changes in RPM may be an indication that preventive maintenance or repair is necessary.

14.4.1.3 If the centrifuge RPM falls outside of the minimum and maximum as set by this protocol, the centrifuge should be taken out of service and the authorized serviced representative for that model should be contacted for service.

14.4.2 **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):
OSBI Forensic Biology Units' Policy Manual **QC_Centrifuge_PC_Log**

Reference(s):
OSBI Criminalistics Services Division Quality Manual and Quality Procedures

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QC_15 pH METER (↑ Table of Contents)

15.1 **Scope**

Quality control for pH meter.

15.2 **Reagents & Supplies**

15.2.1 Deionized Water

15.2.2 pH meter filling solution

15.2.3 0.1M HCl, 0.1M Nitric Acid, 1:10 dilution of household laundry bleach or 0.1-0.5% liquid detergent solution

15.2.4 Electrode storage solution

15.2.5 Standardized reference buffer solutions

15.2.6 Kimwipe or equivalent lint-free cloth

15.3 **Equipment**

15.3.1 pH meter

15.4 **Individual Steps of the Procedure**

15.4.1 **Maintenance Schedule**

15.4.1.1 **Monthly**

15.4.1.1.1 Wipe any salt deposits off electrode.

15.4.1.1.2 Add filling solution to electrode, if necessary.

15.4.1.2 **As Needed**

15.4.1.2.1 Clean electrode; recommended cleaning procedures include one of the following:

15.4.1.2.1.1 Soak electrode in 0.1M HCl or 0.1M Nitric Acid for one-half hour.

15.4.1.2.1.2 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.

15.4.1.2.1.3 Procedure detailed in the operator's manual for that electrode.

15.4.1.2.1.4 Change electrode storage solution.

15.4.1.2.2 After cleaning, drain and refill the reference chamber and soak the electrode in storage solution for at least 1 hour.

15.4.2 **Performance Check**

15.4.2.1 Performance check the pH meter on the day of use, prior to testing samples, as follows:

15.4.2.1.1 Select two standardized reference buffer solutions that bracket the expected pH of the sample to be tested.

15.4.2.1.2 Follow the manufacturer's instructions as listed in the operator's manual.

15.4.2.1.3 If the slope falls within the specifications as listed in the operator's manual, measurement of samples may proceed. Slope may not be available on all pH meters; therefore, this measurement may not be applicable.

15.4.3 **Required Documentation**
Record all maintenance and performance checks performed on the pH Meter Maintenance and Performance Check Log (**QC_pH_Meter_Maint_&_PC_Log**).

15.4.4 **Measurement**

15.4.4.1 Rinse electrode with distilled water and wipe away excess with a tissue.

15.4.4.2 Place the electrode in the sample. Gently swirl sample if needed.

15.4.4.3 When display is stable, record pH.

15.5 **Interpretation of Results (pH Meter Performance)**

15.5.1 The slope must fit within the recommended range as indicated by the user's manual (if applicable).

15.5.2 If the slope falls outside the range specified by the manual, make sure the discrepancy is not due to a problem with the electrode.

15.5.3 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.

15.5.4 If the problem is due to the electrode, the electrode should be replaced, and the faulty electrode properly disposed of.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_pH_Meter_Maint_&_PC_Log**

Reference(s):
Thermo Scientific Orion Star A210 Series Electrochemistry Benchtop Meters (pH meter user guide)

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

QC_16 WATER PURIFICATION SYSTEM (↑ Table of Contents)

16.1 **Scope**

Quality control for water purification system.

16.2 **Reagents & Supplies**

16.2.1 Purification Pack

16.2.2 Sanitization Pack

16.2.3 185/254nm Ultraviolet (UV) lamp

16.2.4 0.2µm Microfilter – Point-of-Use

16.2.5 Biofilter – Point-of-Use

16.3 **Equipment**

16.3.1 ELGA PURELAB flex water purification system

16.4 **Individual Steps of the Procedure**

16.4.1 **Maintenance**

16.4.1.1 **Inlet Filter Cleaning**

16.4.1.1.1 The inlet filter cleaning should be performed two times a year, or as necessary, to ensure that it does not become blocked with particles in the feed water supply, which could affect performance.

16.4.1.1.2 Perform the replacement according to the ELGA PURELAB flex Operator Manual.

16.4.1.2 **Purification Pack Replacement**

16.4.1.2.1 The purification pack should be replaced every 12 months, or as necessary, not to exceed every 5 years.

16.4.1.2.2 Reset the Purification Pack change reminder.

16.4.1.2.3 Perform the replacement according to the ELGA PURELAB flex Quick Start Guide or ELGA Quick Reference Guide.

16.4.1.3 **Sanitization Pack Replacement**

16.4.1.3.1 The sanitization pack should be replaced every 2 years.

16.4.1.3.2 Reset the Sanitization reminder.

16.4.1.3.3 Accept Sanitization and follow the instructions on the Display Handset.

16.4.1.4 **UV Lamp Replacement**

16.4.1.4.1 The UV lamp should be replaced when:

16.4.1.4.1.1 Every 12-18 months, or as necessary, not to exceed every 2 years; or

16.4.1.4.1.2 UV lamp failure occurs (water system will prompt user to change UV lamp);

16.4.1.4.1.3 UV lamp efficiency decreases affecting water purity (water system will prompt user to change UV lamp).

16.4.1.4.2 Perform the replacement according to the ELGA PURELAB flex Operator Manual.

16.4.1.5 **Point-of-Use Filters Replacement**

16.4.1.5.1 The 0.2µm microfilter and biofilter should be replaced every 90 days, or as necessary, not to exceed every 2 years.

16.4.1.5.2 Details of how to install point-of-use filters are supplied with each filter.

16.4.1.6 Document maintenance on the Water Purification System Maintenance Log (**QC_Water_System_Maint_Log**).

16.5 **Interpretation of Results (Water Purification System Performance)**

16.5.1 The expected delivery flowrate maximum at 15°C should be up to 2 l/min.

16.5.2 The expected resistivity at 25°C should be 18.2 MΩ-cm; however, 10 MΩ-cm – 18.2 MΩ-cm is acceptable.

16.5.2.1 If the resistivity is not within the range of 10 MΩ-cm – 18.2 MΩ-cm, then the inlet should be cleaned and the Purification Pack replaced.

16.6 **Notes**

16.6.1 The table below lists the recommended life expectancies for the water system consumables; however, service life is an estimate only and will depend on the application (water system use) and feed water quality.

Description	Typical Service Life	Maximum Shelf Life
Purification Pack	12 months	5 years
Sanitization Pack	N/A	2 years
185/254nm UV Lamp	12-18 months	2 years
0.2µm Microfilter – Point-of-Use	90 days	2 years
Biofilter – Point-of-Use	90 days	2 years

16.6.2 An ELGA trained service technician should carry out any other maintenance not included in this policy, including system calibration, if necessary.

Attachment(s):
OSBI Forensic Biology Units' Policy Manual **QC_Water_System_Maint_Log**

Reference(s):
ELGA PURELAB flex Operator Manual

ELGA PURELAB flex Quick Start Guide

ELGA Quick Reference Guide

OSBI Criminalistics Services Division Quality Manual and Quality Procedures

Approval

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.

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Technical Manager,
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Signature

7/07/23
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7/07/23
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