

TENNESSEE BUREAU OF INVESTIGATION
Forensic Services Division



Forensic Biology Quality Assurance Manual

Tennessee Bureau of Investigation

Forensic Services Division

Forensic Biology Quality Assurance Manual



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1. Scope

ISO/IEC 17025:2017 contains requirements for laboratories to enable them to demonstrate they operate competently, and are able to generate valid results. The ANAB AR 3125 document supplements ISO/IEC 17025:2017 and contains specific accreditation requirements for forensic science testing labs. The FBI Quality Assurance Standards (FBI QAS) describe the quality assurance requirements for laboratories performing forensic DNA testing or using the Combined DNA Index System (CODIS) to ensure the quality and integrity of the data generated by the laboratory. The FBI QAS also apply to vendor laboratories that perform forensic DNA testing in accordance with Standard 17. The Forensic Biology and CODIS Units of the Tennessee Bureau of Investigation (TBI) will adhere to the standards provided in the above documents when performing body fluid identification, nuclear DNA testing, and databasing.

1.1. Goals

- 1.1.1. To provide the users of laboratory services access to body fluid identification and DNA typing of selected biological materials associated with official investigations using Short Tandem Repeat (STR) Polymerase Chain Reaction (PCR) DNA testing.
- 1.1.2. To ensure the quality, integrity, and accuracy of both body fluid identification testing and the DNA typing data and its presentation through the implementation of a detailed Quality Assurance (QA) program.
- 1.1.3. To maintain a CODIS database of convicted offenders, arrestees, and forensic samples.

1.2. Objectives

- 1.2.1. Monitor the analytical testing, reporting procedures, and courtroom presentation for DNA typing by means of Quality Control (QC) standards and proficiency tests.

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- 1.2.2. Verify that the entire DNA typing procedure is operating within the established performance criteria and that the quality and validity of the analytical data are maintained.

- 1.2.3. Ensure that problems are noted and that corrective action is taken and documented.



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2. Terms and Definitions

Accredited laboratory is a DNA laboratory that has received formal recognition that it meets or exceeds a list of standards, including the FBI Director's Quality Assurance Standards, to perform specific tests, by a nonprofit professional association of persons actively involved in forensic science that is nationally recognized within the forensic community in accordance with the provisions of the Federal DNA Identification Act (42 U.S.C. § 14132) or subsequent laws.

Accuracy is the ability of a measurement to give results close to an actual (true) value.

Administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.

Analyst/Forensic Scientist/Examiner (or equivalent role, position, or title as designated by the Assistant Director of Forensic Services) is an employee or contract employee of the laboratory. The job duties of this employee may be casework screening, casework testing, databasing, and/or quality control testing.

Analytical documentation is the documentation of procedures, standards, controls, and instruments used; observations made; results of tests performed; and charts, graphs, photos, and other documentation generated which are used to support the analyst's conclusions.

Analytical procedure is an orderly, step-by-step process designed to ensure operational uniformity and to minimize analytical drift.

Analytical threshold is the minimum height requirement 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.

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Audit is an inspection used to evaluate, confirm, and/or determine the extent to which specified requirements are fulfilled.

Audit team is one or more individuals, including at least one auditor, that perform an inspection of a laboratory. At least one audit team member shall be or have been an analyst previously qualified in the laboratory's current DNA technologies and platforms.

Auditor is an individual who has successfully completed the FBI's DNA auditor training course.

Biochemistry is the study of the nature of biologically important molecules in living systems, DNA replication and protein synthesis, and the quantitative and qualitative aspects of cellular metabolism.

Casework CODIS administrator (or equivalent role, position, or title as designated by the Assistant Director of Forensic Services) is an employee of the laboratory responsible for administration and security of the laboratory's CODIS at a laboratory performing DNA analysis on forensic and casework reference samples.

Casework reference sample is biological material obtained directly from a known individual and collected for purposes of comparison to forensic samples.

Certified reference material is a material for which values are obtained by a technically valid procedure and accompanied by, or traceable to, a certificate or other documentation which is issued by a certifying body (e.g. NIST).

CODIS is the Combined DNA Index System administered by the FBI. CODIS links DNA evidence obtained from crime scenes, thereby identifying serial criminals. CODIS also compares crime scene evidence to DNA profiles from offenders, thereby providing investigators with the identity of the putative perpetrator. In addition, CODIS contains profiles from missing persons, unidentified human remains, and relatives of missing persons. There are three levels of CODIS: the Local DNA Index System (**LDIS**), used by individual laboratories; the State DNA Index System (**SDIS**), used at the state level to serve as a state's DNA database containing DNA profiles from LDIS laboratories; and the National DNA Index System (**NDIS**),

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managed by the FBI as the nation's DNA database containing all DNA profiles uploaded by participating states.

CODIS user is an employee or contract employee who has login access to the CODIS system and is authorized to read, add, modify, and/or delete DNA records in CODIS.

Competency test(s) is a written, oral, and/or practical test or series of tests designed to establish that an individual has demonstrated achievement of technical skills and met minimum standards of knowledge necessary to perform forensic DNA analysis.

Competency is the demonstration of technical skills and knowledge necessary to perform forensic DNA analysis successfully.

Contamination is the unintentional introduction of exogenous DNA into a DNA sample or PCR reaction.

Continuing education is an educational activity (such as a class, lecture series, conference, seminar, or short course) that is offered by a recognized organization or individual that brings participants up-to-date in their relevant area of knowledge.

Contract employee is an individual that performs DNA typing and/or analytical support services to the NDIS participating laboratory. The person performing these services must meet the relevant qualifications for the equivalent position in the NDIS participating laboratory. A contract employee cannot serve as a casework CODIS Administrator or technical leader and cannot be counted as a full-time qualified DNA analyst for purposes of satisfying the definition of a laboratory. Employment of a contract employee by multiple NDIS participating laboratories and/or vendor laboratories shall be disclosed and shall only be permitted subject to approval by the technical leader of the NDIS participating laboratory for which the contract employee is performing DNA typing and/or analytical services.

Control is a sample used to demonstrate that a method works correctly and to ensure the data are valid.

Coursework is an academic class officially recognized and taught through a college or university program in which the participating

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student successfully completed and received one or more credit hours for the class.

Critical equipment or instruments are those requiring calibration, certification, or a performance check prior to use and periodically thereafter.

Critical reagents are those whose performance is vital to the success of the DNA testing and require testing on known samples before use on evidentiary or casework reference samples.

Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic and/or casework reference samples.

Differential amplification is the selection of one target region or locus over another during the polymerase chain reaction. Differential amplification can also arise between two alleles within a single locus if one of the alleles has a mutation within a PCR primer-binding site, causing this allele to be copied less efficiently because of the primer-template mismatch.

DNA record is a database record that includes the DNA profile as well as data required to manage and operate NDIS, i.e., the Originating Agency Identifier, which serves to identify the submitting agency; the Specimen Identification Number; and DNA personnel associated with the DNA profile analyses.

DNA type (also known as a DNA profile) is the genetic constitution of an individual at defined locations (also known as loci) in the DNA. A DNA type derived from nuclear DNA typically consists of one or two alleles at several loci (e.g., short tandem repeat loci).

Employee is a person (1) in the service of the applicable federal, state, or local government, subject to the terms, conditions, and rules of federal, state, or local employment and eligible for the federal, state, or local benefits of service; or (2) formerly in the service of a federal, state, or local government who returns to service in the agency on a part-time or temporary basis. For purposes of a vendor laboratory, an employee is a person in the service of a vendor laboratory and subject

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to the applicable terms, conditions, and rules of employment of the vendor laboratory.

Expert System is a software program or set of software programs designed to interpret single source DNA data in accordance with laboratory defined quality assurance rules and identify DNA data not satisfying laboratory defined quality assurance rules, without human intervention.

FBI is the Federal Bureau of Investigation, the federal agency authorized by the DNA Identification Act of 1994 to issue quality assurance standards governing forensic DNA testing laboratories and to establish and administer the National DNA Index System (NDIS).

Forensic DNA analysis is the process of detection, identification and evaluation of biological evidence in criminal matters using DNA technologies.

Forensic sample is a biological sample originating from and associated with a crime scene. For example, a sample associated with a crime scene may include a sample that has been carried away from the crime scene.

Genetics is the study of inherited traits, genotype/phenotype relationships, and population/species differences in allele and genotype frequencies.

Guidelines are a set of general principles used to provide direction and parameters for decision making.

Inconclusive is a determination that no inclusion or exclusion can or could be drawn from the comparison of a casework reference sample to a forensic sample. An inconclusive result could be due to uninterpretable data/profile.

Integral component is that portion of an academic course that is so significant and necessary to the understanding of the subject matter as a whole that the course would be considered incomplete without it.

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Internal validation is the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

Known samples are biological material whose identity or type is established.

Laboratory is a facility employing at least two full-time employees who are qualified DNA analysts **and** having and maintaining the capability to perform the DNA analysis of forensic samples and/or casework reference samples at that facility.

Laboratory support personnel (or equivalent role, position, or title as designated by the Assistant Director of Forensic Services) are employees or contract employees who perform laboratory duties exclusive of analytical techniques on forensic or database samples.

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 the process. The same method may be conducted using different equipment (automated vs manual) when appropriately validated.

Methodology refers to the categories of methods used to perform a step of a DNA-typing technology: for example, extraction methods (manual vs. automated), quantification methods (slot blot, fluorometry, real-time); typing test kit; and platform (capillary electrophoresis, real-time gel and end-point gel systems).

Molecular biology is the study of the theories, methods, and techniques used in the study and analysis of gene structure, organization, and function.

Multi-laboratory system is used to describe an organization that has more than one laboratory performing forensic DNA analysis.

Multiplex system is a test providing for simultaneous amplification of multiple loci that is either prepared commercially or by a laboratory.

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Negative amplification control is an analytical control used to detect DNA contamination of the amplification reagents. This control consists of only amplification reagents without the addition of template DNA.

NIST is the National Institute of Standards and Technology.

Nonconformity is not meeting, implementing, maintaining, or complying with one or more of the requirements of accreditation standards, laboratory's policies, procedures, or other quality system documents.

On-site visit is a scheduled or unscheduled visit to the vendor laboratory work site by one or more representatives of an NDIS participating laboratory who is (are) a qualified or previously qualified DNA analyst(s) in the technology, platform and typing amplification test kit used to generate the DNA data, or designated FBI employee(s), to assess and document the vendor laboratory's ability to perform analysis on outsourced casework.

Outsourcing is the utilization of a vendor laboratory to provide DNA services in which the NDIS participating laboratory takes or retains ownership of the DNA data for entry into CODIS, when applicable. Outsourcing does not require the existence of a contractual agreement or the exchange of funds.

Ownership occurs when any of the following criteria are applicable:

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

Ownership review is the technical review of outsourced DNA data required by Standard 17 of the FBI Quality Assurance Standards (QAS). This review is to be distinguished from the technical and administrative review reviews required by Standard 12 of the FBI QAS.

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For outsourced DNA data, the vendor laboratory is responsible for conducting the technical and administrative reviews required by Standard 12 of the FBI QAS.

Performance check is a quality assurance measure to assess the functionality of critical laboratory instruments and equipment.

Platform is the type of analytical system utilized to generate DNA profiles, such as capillary electrophoresis, real-time gel, and end-point gel instruments or systems.

Polymerase Chain Reaction (PCR) is an enzymatic process by which a specific region of DNA is replicated during repetitive cycles, which consist of the following:

1. Denaturation of the template;
2. Annealing of primers to complementary sequences at an empirically determined temperature; and
3. Extension of the bound primers by a DNA polymerase.

Positive amplification control is an analytical control sample that is used to determine if the PCR performed properly. This control consists of the amplification reagents and a known DNA sample.

Precision *characterizes* the degree of mutual agreement among a series of individual measurements, values, and/or results.

Preferential amplification is the unequal sampling of the two alleles present in a heterozygous locus.

Procedure (protocol, standard operating procedure, or other equivalent) is an established practice to be followed in performing a specified task or under specific circumstances.

Proficiency testing is a quality assurance measure used to monitor performance and identify areas in which improvement may be needed. Proficiency tests may be classified as:

1. An internal proficiency test, which is produced by the agency undergoing the test.
2. An external proficiency test, which may be open or blind, is a test obtained from an approved proficiency test provider.

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Qualified is an adjective used to describe an individual who meets the requirements for the position, has successfully completed the laboratory's training requirements, and is authorized to perform a specific task or role.

Qualified auditor is a current or previously qualified DNA analyst who has successfully completed the FBI's DNA auditor training course.

Qualitative statement is a description of the evidence (e.g. partial profile, mixture profile) or a conclusion of any comparisons that were performed without statistical significance provided (e.g., consistent with an intimate sample)

Quality system is the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management.

Quantitative PCR is a method of determining the concentration of DNA in a sample by use of the polymerase chain reaction.

Quantitative statement is a conclusion that provides a statistical measure of the comparison performed.

Reagent is a substance or mixture of substances in the analytical process to detect, measure, produce, or interact with other substances.

Reagent blank control is an analytical control sample that contains no template DNA and is used to monitor contamination from extraction to final fragment or sequence analysis. This control is treated the same as, and parallel to, the forensic and/or casework reference samples being analyzed.

Reference material (certified or standard) is a material for which values are certified by a technically valid procedure and accompanied by, or traceable to, a certificate or other documentation which is issued by a certifying body.

Reproducibility is the ability to obtain the same result when the test or experiment is repeated.

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Review is an evaluation of documentation to check for consistency, accuracy, and completeness.

Second agency is an entity or organization external to and independent of the laboratory.

Semiannual is used to describe an event that takes place two times during one calendar year. For requirements specified by the QAS as semiannual, the first event takes place in the first six months of that year and the second event takes place in the second six months of that year, and the interval between the two events is at least four months and not more than eight months.

Service is the performance of those adjustments or procedures specified which are to be performed by the user, manufacturer, or other service personnel in order to ensure the intended performance of instruments and equipment.

Stochastic threshold is the peak height value below which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele in a heterozygous pair may have occurred.

Technical leader (or equivalent role, position, or title as designated by the Assistant Director of Forensic Services) is an employee who is accountable for the technical operations of the laboratory and who is authorized to stop or suspend laboratory operations.

Technical review is an evaluation of reports, notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions.

Technical reviewer is an employee or contract employee who is a current or previously qualified analyst in the methodology being reviewed that performs a technical review of, and is not an author of, the applicable report or its contents.

Technician (or equivalent role, position, or title as designated by the Laboratory Director) is an employee or contract employee who performs analytical techniques on forensic samples under the supervision of a qualified analyst. Technicians do not interpret data, reach conclusions on typing results, or prepare final reports.

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Technology is used to describe the type of forensic DNA analysis performed in the laboratory, such as RFLP, STR, YSTR, or mitochondrial DNA.

Test kit is a preassembled set of reagents that allows the user to conduct a specific DNA extraction, quantification, or amplification.

TOMIS (Tennessee Offender Management Information System) is the management system designed to monitor inmates and/or individuals on parole within the Tennessee Corrections Department.

Traceability is the property of a result of a measurement whereby it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

Validation is a planned process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes the following:

1. Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.
2. Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

Vendor laboratory is a governmental or private laboratory that provides DNA analysis services to another laboratory or agency and does not take ownership of the DNA data for purposes of entry into CODIS.

Work product is the material that is generated as a function of analysis, which may include extracts, amplified product, and amplification tubes or plates as defined by the laboratory.



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3. Quality Assurance Program

Elements 3.1 through 3.17 below are elements of the quality system that the TBI laboratory documents in various manuals/documents such as the Division Quality Assurance Manual, the Forensic Biology Policies and Procedures Manual, the Forensic Biology: STR DNA Typing Manual, the Forensic Biology Quality Assurance Manual, and the CODIS Manual.

- 3.1. **Goals and objectives** must define, establish, or reference the goals and objectives for the laboratory.
- 3.2. **Organization and management** must define, establish, or reference the organization and management structure of the laboratory, the interrelationship of the various DNA positions, as well as the responsibilities of personnel.
- 3.3. **Personnel** must define, establish, or reference the training and qualifications required for each position within the laboratory and describe the continuing education program for the laboratory.
- 3.4. **Facilities** must define, establish, or reference the laboratory's practices or procedures for laboratory security and its approach for maintaining the integrity of DNA analyses and evidence examination.
- 3.5. **Evidence control** must define, establish, or reference the laboratory's procedures for handling and preserving evidence as well as the laboratory's definitions for what constitutes work product and evidence.
- 3.6. **Validation** must define, establish, or reference the practices and procedures for implementing new methods used by the laboratory and the process for incorporating those new procedures.
- 3.7. **Analytical procedures** must define, establish, or reference the use of current and approved standard operating procedures for validated methods.

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- 3.8. **Equipment calibration and maintenance** must define, establish, or reference the laboratory's program for conducting performance checks and calibrations of equipment and instruments and the laboratory must maintain a list of its critical instruments and/or equipment.
- 3.9. **Reports** must define, establish, or reference the laboratory's procedure for how it maintains its case files, how it generates its laboratory reports, and its policy for describing how the laboratory maintains confidentiality and privacy when applicable to reports, case files, and DNA records and databases.
- 3.10. **Review** must define, establish, or reference how the laboratory performs its technical and administrative review of all case files, the qualifications of personnel who perform reviews, review procedures associated with the upload of DNA data, as well as include a documented program for the annual testimony monitoring of its analysts.
- 3.11. **Proficiency testing** must define, establish, or reference the laboratory's program for administering external proficiency tests to DNA personnel to the full extent in which they participate in casework.
- 3.12. **Corrective action** must define, establish, or reference the laboratory's process for corrective action in casework and proficiency testing.
- 3.13. **Audits** must define, establish, or reference the laboratory's program for participation in internal and external DNA audits.
- 3.14. **Safety** must define, establish, or reference the laboratory's safety program.
- 3.15. **Outsourcing** must define, establish, or reference the laboratory's procedures for outsourcing samples and ensuring the integrity of those samples.

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3.16. Document Retention

The document retention policy is addressed in the division Quality Assurance Manual and is summarized below:

- 3.16.1. Proficiency Tests are retained for time of employment plus five years.
- 3.16.2. Personnel Training Records are retained for time of employment plus five years.
- 3.16.3. Audit Records are retained for one accreditation cycle.
- 3.16.4. Corrective Actions are retained for one accreditation cycle.
- 3.16.5. Continuing Education is retained for time of employment plus five years.
- 3.16.6. Court Testimony Monitoring forms are retained for time of employment plus five years.
- 3.16.7. Case files are retained according to statute requirements for case records.
- 3.16.8. CODIS sample receipt and processing records- refer to the CODIS Manual
- 3.16.9. CODIS hit confirmation- refer to the CODIS Manual
- 3.16.10. CODIS sample retention- refer to the CODIS Manual

- 3.17. The quality system as applicable to DNA will be reviewed annually under the direction and documented approval of the Technical Leader. This review will be independent of the audit required by Standard 15 of the Quality Assurance Standards document and will include a review of the quality manual, training manual, and procedures

4. Organization and Management

- 4.1. The name and position of the members of the Forensic Biology Unit can be found in the Organizational Chart located on the TBI Intranet. An outline of the organizational structure of the TBI Forensic Biology Unit is presented below:
 - TBI Director
 - Assistant Director of Forensic Services



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- Forensic Quality Assurance Manager (QA Manager)
- DNA Technical Leader
- DNA Supervisor/Laboratory Regional Supervisor
- DNA Analyst 2 (Forensic Scientist 2)
- DNA Analyst 1 (Forensic Scientist 1)
- CODIS Administrator (see CODIS protocol for responsibilities)
- CODIS (Forensic) Technician
- Contract Employee

4.2. Functional Responsibility

4.2.1. *Assistant Director of Forensic Services*- Coordinates training and continuing education of DNA personnel. Coordinates administrative and fiscal activities.

4.2.2. *Quality Assurance Manager*- Under executive direction, is responsible for administering a quality assurance and safety program for the laboratory.

4.2.3. *DNA Technical Leader*-is an employee who is accountable for the technical operations of the laboratory and who is authorized to stop or suspend laboratory operations. Other duties include:

- Oversee the technical operations of the laboratory.
- Authority to initiate, suspend and resume DNA analytical operations for the laboratory or an individual.
- To evaluate and document approval of all validations and methods used by the laboratory and to propose new or modified analytical procedures to be used by analysts.
- To review the academic transcripts and training records for newly qualified analysts and approve their qualifications prior to independent casework analysis and document such review.
- To approve the technical specifications for outsourcing agreements.

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- To review internal and external DNA Audit documents and, if applicable, approve corrective action(s), and document such review.
- To review, on an annual basis, the procedures of the laboratory and document such review.
- To review and approve the training, quality assurance and proficiency testing programs in the laboratory.
- To review requests by contract employees for employment by multiple NDIS participating and/or vendor laboratories and, if no potential conflict of interests exist, may approve such requests.
- Conducts semiannual on site visits to regional laboratories.

NOTE:

In the event the DNA Technical Leader position should become vacant, the Assistant Director in charge of the Forensics Services Division will appoint an interim DNA Technical Leader from one of the TBI's three regional DNA laboratories. This DNA analyst must satisfy the requirements of Standard 5 of the DNA Quality Assurance Standards Document. This appointment will remain in effect until an official appointment is made.

In the event the DNA Technical Leader position should become vacant, and a replacement satisfying the requirements of Standard 5 of the DNA Quality Assurance Standards Document cannot be identified internally, the laboratory must submit a contingency plan to the FBI. This plan must be submitted to the FBI within 14 days of the vacancy using Appendix B from the DNA QAS document. Laboratory work already in progress may be completed over this time-frame. **No new laboratory work will be initiated during this time-frame until the contingency plan is approved by the FBI.**

4.2.4. DNA Supervisor/Regional Laboratory Supervisor

- Supervises operations of the DNA laboratory such as scheduling assignments and case reviews.
- Coordinates Peer Review processes.

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- Maintains supplies, equipment, and other materials necessary for the operation of the section.
- Enforces policy and procedure for operations and functions within the sphere of authority.

4.2.5. DNA Analyst (Forensic Scientist)

- Performs analysis, documentation, interpretation, and reporting of casework.
- Provides expert testimony.
- May gather physical and biological evidence at crime scenes.
- Participates in Peer Review processes.
- Maintains technical and professional competence.
- Conducts troubleshooting and method development.
- Ensures compliance with safety rules and regulations.
- Prepares reagents.
- Conducts laboratory housekeeping.

4.2.6. CODIS Administrator (see CODIS protocol for responsibilities)

4.2.7. CODIS (Forensic) Technician

- Receives Convicted Offender and/or Arrestee Samples.
- Assigns identification numbers and logs each sample into a computerized tracking system.
- Prepares dried stain cards of each sample submitted if applicable.
- Enters data into the Tennessee Offender Management Information System (TOMIS).
- Helps verify data entered into the Convicted Offender DNA Index System (CODIS).
- Establishes and maintains personal contacts with Department of Corrections and law enforcement personnel.

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- Assists with general laboratory tasks when necessary.
- Ensures compliance with safety rules and regulations.

4.2.8. *Contract Employee*- is an individual that may perform serological screening, DNA typing and/or analytical support services to the laboratory. The person performing these services must meet the relevant qualifications for the equivalent position in the laboratory.

4.3. If a laboratory has the number of qualified analysts fall below two full time employees, qualified analyst(s) from one of TBI's other regional laboratories may be transferred to that laboratory. If no qualified analysts are available for transfer, no new DNA testing will be initiated. DNA casework already in progress may be completed. The completed work will be technically and administratively reviewed by a qualified analyst in one of the other TBI regional laboratories. New DNA casework may be transferred to one of the TBI's regional laboratories for testing.

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5. Personnel

5.1. Job Descriptions

5.1.1. DNA Supervisor

- Supervises the examination of physical evidence.
- Supervises chemical tests for the identification and analysis of blood and other body fluids.
- Testifies in court as an expert witness on laboratory findings.
- Maintains supplies, equipment, and other materials necessary for the operation of the unit.
- Assigns, trains, supervises, and evaluates subordinate staff and their work.
- Enforces policy and procedure for operations and functions within his/her sphere of authority.
- Prepares, checks, and reviews important detailed and complex laboratory records.

5.1.2. DNA Technical Leader

- Oversees the technical operations of the DNA laboratories.
- Authority to initiate, suspend and resume DNA analytical operations for the laboratory or an individual.
- Evaluates and documents approval of all validations and methods used by the DNA laboratory and to propose new or modified analytical procedures to be used by analysts.
- Reviews the academic transcripts and training records for newly qualified DNA analysts and technical reviewers. Documents the review and approval of their qualifications prior to independent casework analysis and technical reviews.
- Approves the technical specifications for outsourcing agreements.
- Reviews internal and external DNA Audit documents and, if applicable, approves corrective action(s), and documents such review.

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- Reviews, on an annual basis, the procedures of the DNA laboratory and documents such review.
- Reviews and approves the training, quality assurance and proficiency testing programs in the DNA laboratory.
- Reviews requests by DNA contract employees for employment by multiple NDIS participating and/or vendor laboratories and, if no potential conflict of interests exists, may approve such requests.
- Conducts semiannual on site visits to the regional DNA laboratories

5.1.3. DNA Analyst 2 (Forensic Scientist 2)

- Analyzes and examines physical evidence.
- May gather physical and biological evidence at crime scenes.
- Analyzes and examines biological or physical evidence to identify and characterize blood and other body fluids and to determine their nature, type, or origin.
- Composes technical laboratory reports describing the evidence examined, the results of examinations, and interpretation of results.
- Testifies in criminal court as an expert witness and provides expert opinion testimony.
- May lead others in work as assigned.
- Conducts peer review on case records.

5.1.4. DNA Analyst 1 (Forensic Scientist 1)

- Analyzes and examines biological or physical evidence to identify and characterize blood and other body fluids and to determine their nature, type, and origin under direct supervision.
- May gather physical and biological evidence at crime scenes.
- Learns to compose technical laboratory reports describing the evidence examined, the results of examinations, and interpretation of results.
- Learns to testify in criminal court as an expert witness and provide expert opinion testimony.



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- May perform all stages of DNA analysis on known and/or duplicate samples.
- May perform all stages of DNA analysis after completion of the training period.
- May perform the job description of a DNA analyst 2 upon completion of the training period.

5.1.5. CODIS (Forensic) Technician

- Receives, preserves, and tracks submitted Convicted Offender and/or Arrestee samples.
- Maintains all CODIS information of Convicted Offender and/or Arrestee samples.
- Communicates with Convicted Offender and/or Collection sites across the state.
- Performs general laboratory tasks whenever needed.
- Refer to the CODIS Protocol for additional job duties.

5.1.6. Contract Employee

- May perform duties described in 5.1.3 and/or 5.1.4.

5.2. Scientists who have successfully completed training and are authorized to perform, report, and review casework and/or CODIS verifications are authorized to develop, modify, verify, and validate methods under the direction of the technical leader. Scientists who have completed training in quality control or have completed competency testing with mock samples are authorized to assist a qualified scientist with validations.

5.3. The technical leader and supervisors are authorized to create, revise, and review policies in ensur for Forensic Biology documents. The technical leader, supervisors, and casework CODIS administrators are authorized to create, revise, and review policies in ensur for CODIS documents.

5.4. Any scientist who has successfully completed training is authorized to act as a training officer for the area(s) in which he/she was previously trained.



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5.5. Qualifications

5.5.1. Qualifications will be based on the hire or promotion date when determining the applicable version of the Quality Assurance Standards for education, experience, and training requirements.

5.5.2. DNA Supervisor

- Education – Must have, at a minimum, a Bachelor's degree or its equivalent in biology, chemistry, forensic science, and/or other forensic science related area, including a minimum of 24 semester or equivalent credit hours in chemistry and college coursework or classes covering the areas of: genetics, biochemistry, and molecular biology (e.g. molecular genetics, recombinant DNA technology), and statistics or population genetics. Analysts qualified after issuance of the audit document (July 1, 2004) must have a minimum of 6 semester or equivalent hours covering the required subject areas. After July 1, 2009, analysts must have a minimum of 9 semester or equivalent hours addressing the required coursework.
- Experience – Must have either four (4) years of full-time professional forensic science work or additional graduate coursework in a natural or physical science that may be substituted for the required experience on a year-for-year basis to a maximum of one year.
- Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Literature distribution will be documented by reading current scientific literature. Literature distribution will be documented by either hard copy or electronically. Must attend seminars, webinars, courses, or

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professional meetings at least once (1) a year. The Technical Leader and all qualified TBI DNA analysts must have a minimum of 8 hours of continuing education completed by the end of a calendar year.

5.5.3. DNA Technical Leader

- **Education** – Must have, at a minimum, a Master's degree in biology, chemistry, or a forensic science-related area (including 24 semester credits or its equivalent of chemistry) and successfully completed a minimum of 12 semester or its equivalent credit hours of a combination of undergraduate and graduate course work or classes covering the subject areas of biochemistry, genetics, molecular biology (e.g. molecular genetics, recombinant DNA technology), and statistics or population genetics. If appointed or hired after July 1, 2009, the Technical Leader must have four separate courses. The 12 semester or equivalent credit hours must include, at a minimum, one graduate level class registering three or more semester or equivalent credit hours.
- **Experience** – Must have a minimum of three (3) years of forensic DNA laboratory experience. Beginning July 1, 2009, experience must be in human DNA as a qualified analyst on forensic samples. Prior to July 1, 2009, the Technical Leader should have successfully completed the DNA Auditing Workshop sponsored by the FBI. After July 1, 2009, the FBI DNA auditor training must be successfully completed within one year of appointment, if not previously completed. The technical leader shall be a current or previously qualified analyst in each technology utilized by the laboratory, or have documented training in each



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technology utilized in the laboratory within one year of appointment.

- Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Literature distribution will be documented by either hard copy or electronically. Must attend seminars, webinars, courses, or professional meetings at least once (1) a year. The Technical Leader and all qualified TBI DNA analysts must have a minimum of 8 hours of continuing education completed by the end of a calendar year.

5.5.4. DNA Analyst 2 (Forensic Scientist 2)

- Education – Must have, at a minimum, a B.A./B.S. degree or its equivalent in biology, chemistry, or a forensic science-related area (including a minimum of 24 semester or equivalent credit hours in chemistry) and college coursework or classes covering the areas of: genetics, biochemistry, and molecular biology (e.g. molecular genetics, recombinant DNA technology), and statistics or population genetics. Analysts qualified after issuance of the audit document (July 1, 2004) must have a minimum of 6 semester or equivalent hours covering the required subject areas. After July 1, 2009, analysts must have a minimum of 9 semester or equivalent hours addressing the required coursework.
- Training – Must have, at a minimum, training in the fundamentals of forensic serology and training in DNA analysis provided by individuals, agencies, or other laboratories in a program that includes the methods, procedures, equipment, and materials used in forensic DNA analysis and their applications and limitations.

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- Experience – Prior to any reporting on DNA casework samples, the analyst must have a minimum of six (6) months of forensic serology/DNA experience to include the successful analysis of a range of samples typically encountered in forensic casework, and a working command of each stage of the analysis. The analyst must have knowledge of the scientific principles, techniques, and literature on DNA typing as demonstrated by course work and/or written and/or oral examination. The analyst must also successfully complete a moot case and moot court peer evaluation. Practical laboratory skills in the performance of DNA analysis as demonstrated by observation and successful analytical results and competency in DNA analysis as demonstrated by the successful completion of competency testing designed to evaluate technical and interpretational skills must be demonstrated.

- Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Literature distribution will be documented by either hard copy or electronically. Must attend seminars, webinars, courses, or professional meetings at least once (1) a year. The Technical Leader and all qualified TBI DNA analysts must have a minimum of 8 hours of continuing education completed by the end of a calendar year.

5.5.5. DNA Analyst 1 (Forensic Scientist 1)

- Personnel involved in performing analytical techniques related to DNA analysis may not interpret DNA typing results, prepare final reports, or provide testimony prior to qualification as a DNA Analyst.

- Education– Must have, at a minimum, a B.A. /B.S. degree or its equivalent in biology, chemistry, or a

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forensic science-related area (including a minimum of 24 semester or equivalent credit hours in chemistry). Must complete college coursework or classes covering the areas of: genetics, biochemistry, and molecular biology (e.g. molecular genetics, recombinant DNA technology), and statistics or population genetics prior to qualification as a DNA analyst. Analysts qualified after issuance of the audit document (July 1, 2004) must have a minimum of 6 semester or equivalent hours covering the required subject areas. After July 1, 2009, analysts must have a minimum of 9 semester or equivalent hours addressing the required coursework.

- Training – Must have, at a minimum, training in the fundamentals of forensic serology and training in DNA analysis with individuals, agencies or other laboratories in a program that includes the methods, procedures, equipment and materials used in forensic DNA analysis and their applications and limitations.
- Experience – Prior to performing any independent analytical techniques on forensic cases, the analyst must successfully complete and document a qualifying test (i.e.-moot court). The lab must document the qualification date. Also, the analyst must have a minimum of six (6) months of forensic serology/DNA experience to include the successful analysis of a range of samples typically encountered in forensic casework. The analyst must demonstrate a working command of each stage of the analysis, knowledge of the scientific principles, techniques, and literature on DNA typing as demonstrated by course work and written and/or oral examination. Practical laboratory skills in the performance of DNA analysis as demonstrated by observation and successful analytical results will be evaluated and

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competency in DNA analysis as demonstrated by the successful completion of competency testing designed to evaluate technical and interpretational skills must be completed.

- Continuing Education – Must stay abreast of developments within the field of DNA typing by reading current scientific literature. Literature distribution will be documented by either hard copy or electronically. Must attend seminars, webinars, courses, or professional meetings at least once (1) a year. The Technical Leader and all qualified TBI DNA analysts must have a minimum of 8 hours of continuing education completed by the end of a calendar year.

5.5.6. CODIS (Forensic) Technician

- Education – Education equivalent to graduation from a standard high school
- Training – On-the-job training will be provided.

5.5.7. CODIS Administrator (see CODIS protocol for responsibilities and education/training requirements)

5.5.8. *Contract Employee*-is an individual that may perform DNA typing and/or analytical support services to the laboratory including technical and administrative reviews. The person performing these services must meet the relevant qualifications for the equivalent position in the laboratory. A contract employee cannot serve as a casework CODIS Administrator or technical leader and cannot be counted as a full-time qualified DNA analyst for purposes of satisfying the definition of a laboratory. Employment of a contract employee by multiple NDIS participating laboratories and/or vendor laboratories shall be disclosed and shall only be permitted subject to approval by the technical leader.

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6. Facilities

- 6.1. TBI DNA Laboratory facilities will be such as to permit the correct performance of forensic DNA examinations. The laboratory will ensure that the environmental conditions do not adversely affect the quality required of any examination. Any environmental conditions that can affect the results of examinations will be documented in the appropriate standard operating procedure. All examinations require normal laboratory environmental conditions unless noted in a standard operating procedure. When sampling and/or examinations are undertaken at sites other than a permanent TBI Laboratory facility, the laboratory will ensure that the requirements related to facilities and environmental conditions are met.
- 6.2. If environmental conditions affect the quality of an examination, Forensic Biology unit will monitor, control and record those conditions as required by a standard operating procedure. Examinations will be stopped when the environmental conditions jeopardize the results.
- 6.3. DNA Laboratory units will be responsible for maintaining effective separation between incompatible activities to prevent cross-contamination. Evidence examinations, DNA extractions, quantifications, and PCR setup shall be conducted at separate times or in separate spaces from each other. Amplified product, including real time PCR, shall be generated, processed and maintained in a room separate from the evidence examination, DNA extraction and PCR setup areas. The doors between rooms containing amplified DNA and other areas shall remain closed. Written procedures for cleaning and decontaminating facilities and equipment are maintained on the document control system and/or in the appropriate laboratory area.
 - 6.3.1. The floors in the unit except for the post-amp room will be maintained by contract cleaners. The counters and equipment in the exam rooms will be maintained by the analysts working in these areas. The counters and equipment will be cleaned as needed using 10%

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bleach commercial disinfectants, and/or isopropanol. The extraction, PCR, and quantification set-up hoods and equipment will be cleaned on a monthly basis by an assigned analyst using either 10% bleach and/or isopropanol. In addition to monthly cleaning, each analyst should clean and decontaminate as needed. The post-amp room should be cleaned on a monthly basis. The counters and equipment should be wiped down with isopropanol and/or 10% bleach if contaminated. The post amp room floors should be mopped with a disinfectant solution. All equipment used to clean the post-amp room will remain in the room. Areas which have not been used during the month would not necessarily require cleaning.

- 6.4. Access to and use of all examination areas in the Forensic Biology Laboratory is controlled and limited.
- Visitors do not have unrestricted access to the operational areas of the Forensic Biology Laboratory.
 - All Forensic Biology Laboratory exterior entrance/exit points have proximity badge readers or a lock system to enforce security.
 - Internal areas requiring limited/controlled access have proximity badge readers or a lock system to enforce security.
 - Distribution of access keys and TBI laboratory badges is limited to those individuals approved by the TBI Assistant Director of Forensic Services.
 - The Forensic Biology Laboratory is monitored during vacant hours by intrusion alarms and the TBI Uniformed Officer unit (TBIHQ building only).
 - Evidence storage areas are secured using intrusion detection and lock systems. The storage conditions are such as to prevent loss, deterioration and contamination as well as to maintain the integrity of the evidence. These conditions apply both before and after examinations have been performed.



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7. Evidence Control

7.1. Evidence Handling

- Primary Evidence Vault (PEV) - The laboratory's main evidence storage vault located in the Evidence Receiving Unit.
- Unit Bulk Storage Location (UBSL) (If applicable) – This locked area located in the Forensic Biology unit is accessible by analysts in the Forensic Biology Unit.
- Personal Storage Location (PSL) – These locked areas describe both workstation storage and unit vault personal storage (if applicable) and can only be accessed by unit supervisor and the individual they are assigned.

7.1.1. Evidence must be collected, received, handled, sampled, and stored to preserve the identity, integrity, condition, and security of the item.

7.1.2. Items received by the analyst are kept in the PSL or UBSL in the Forensic Biology unit in a sealed condition. This may be a convenience seal such as a stapled package or a box with its lid applied. These areas remain locked at all times and contain storage lockers and refrigerators. The storage area is only accessible by card-key, combination lock, and/or key entry. Only analysts with items stored in this area and the Unit Supervisor have access. The UBSL may also contain freezers/refrigerators for evidence storage.

7.1.3. Case file folders/evidence for single/multi-section cases are to be picked up and returned to the evidence window. The official chain-of-custody will be maintained electronically. The evidence is then placed in the analyst's PSL or in the UBSL. Each analyst will remove his/her evidence from their PSL or the UBSL as needed. Each work area has lockable storage for evidence currently being examined. Unattended

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evidence undergoing examination will be secured via limited card key access to the unit. When analysis is complete, the analyst should return the sealed evidence to the Forensic Technician in the Evidence Receiving Unit.

- 7.1.4. Only one case is opened at a time per analyst. However, more than one case can be received at a time from a Forensic Technician. Once the case is opened, all items associated with the case are labeled with laboratory and exhibit numbers (if applicable) unique to that case. This is done to make sure that identity, integrity, and security of the evidence samples and paperwork for each case are not compromised.
- 7.1.5. Bloodstain cards will be stored with all other evidence in the case, until they are returned to the submitting agency. Blood tubes will be destroyed after six (6) months. The case file will bear a date of destruction. If the blood tube is shared with toxicology, the case file shall reference toxicology for the date of destruction. If further testing is indicated on any case, then the evidence of that case will be retrieved and maintained as above. The analyst will then complete the examination. The sealed evidence will then be returned to a Forensic Technician and/or forwarded to another laboratory for further testing as needed.
- 7.1.6. All analysts will maintain custody of their samples by sealing and storing in PSL or UBSL. Completed evidence should be returned to the PEV for storage. Seals will be marked with the analyst's initials.



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8. Validation of Analytical Procedures

8.1. Validation is the process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis. It is the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected. A summary of each validation study will be maintained with the data.

8.1.1. Developmental Validation of a DNA Analysis Procedure

- During the development of a DNA procedure and prior to the adoption of the procedure by the DNA laboratory, validation studies must have been conducted by the scientific community. These validation studies form the basis for evaluating the validity, accuracy, precision and reproducibility of a particular DNA procedure.

8.1.2. Developmental validation and scientific literature shall include the following, where applicable:

- Characterization of the genetic marker,
- Species specificity,
- Sensitivity studies,
- Stability studies,
- Reproducibility,
- 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.

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- 8.1.3. Peer-reviewed publication of the underlying scientific principle(s) of a technology shall be required.
- 8.2. Prior to implementing a new DNA procedure that meets the developmental criteria; the TBI Forensic Biology unit will validate the procedure internally to demonstrate reliability of the procedure. Internal validation studies shall be documented and summarized. The technical leader shall approve the internal validation studies. Written documentation for each validation study will be maintained for future reference in each regional laboratory.
- 8.2.1. The internal validation studies shall include as applicable the following:
- Known and non-probative evidence samples or mock evidence samples
 - Reproducibility and precision
 - Sensitivity and stochastic studies
 - Mixture studies
 - Contamination assessment.
- 8.3. Except as provided in Standard 8.3.1 below, internal validation of all manual and robotic methods shall be conducted by each laboratory and reviewed and approved by the laboratory's technical leader prior to using a procedure for forensic applications. The appropriate sample number and type must be used to demonstrate the reliability and potential limitations of the method.
- 8.3.1. Internal validation data may be shared by all locations in the multi-laboratory system. Each laboratory in the multi-laboratory system shall complete, document and maintain applicable precision, sensitivity, and contamination assessment studies. The summary of the validation data shall be available at each site.
- 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.



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

8.3.3. A complete change of detection platform or typing test kit shall require internal validation studies.

- 8.4. Before the introduction of a methodology into casework, an analyst shall successfully complete a competency test to the extent of his or her participation in casework analyses.
- 8.5. The performance of a modified procedure shall be evaluated by comparison with the original procedure using similar DNA samples and the evaluation documented. The evaluation will be reviewed and approved by the technical leader prior to implementation of the modified procedure into casework applications.
- 8.6. Each additional critical instrument shall require a performance check. Modifications to an instrument that do not affect the analytical portion of the instrument shall require a performance check.
- 8.7. Modifications to software, such as an upgrade, shall require a performance check prior to implementation. New software or significant software changes that may impact interpretation or the analytical process shall require a validation prior to implementation.
- 8.8. Validation of all procedures will also adhere to the guidelines outlined in the Division Quality Assurance manual.
- 8.9. Newly validated DNA methods, typing test kit or platform instrument model will be checked against an appropriate and available certified reference material (or sample made traceable to the certified reference material) prior to implementation of the method for casework or database analysis.

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9. Analytical Procedures

- 9.1. The Forensic Biology unit shall have and follow standard operating procedures for each analytical method used by the laboratory.
- 9.2. The start date (beginning of note taking) and stop date (initial draft completion) for testing shall be documented on the first page of the case notes. The stop date will signify that the scientist has reviewed and authorized the results prior to technical review. Any changes or amendments made to the case file after the stop date will require the scientist to initial and date the change, addition, or amendment.
- 9.3. The active examination of a case should not exceed ninety (90) days. If active examination exceeds 90 days, an explanation should be documented in the case file.
- 9.4. Analysis of evidence and evidence samples shall be conducted to provide the maximum information with the least consumption of the sample (the maximum amount of the evidence is returned to the submitting agency). A sample may be consumed to achieve the maximum information. If this occurs, it should be documented in the case file. The analyst must use critical judgment concerning which analytical procedures are appropriate given the constraints of the evidence.
- 9.5. DNA extracts generated from casework evidence are considered evidence and will be retained by the TBI Crime Laboratory. The extracts will be stored frozen in a freezer capable of at least -20°C. The extracts will be retained indefinitely by the TBI unless returned to the submitting agency or an entity approved by the submitting agency. Extracts will be tracked in the Laboratory Information Management System (LIMS). Each extract will be considered in the possession of the scientist during the preparation and testing of the extract. At the completion of testing, the scientist will transfer the extract from their possession to the Forensic Biology Freezer Storage. Dilutions of an extract are not considered evidence and will not be retained.

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- 9.6. CODIS verification extracts are considered work product and will not be retained after completion of testing.
- 9.7. Sample cuttings used in testing are considered work product and may not be retained after completion of testing. When a swab is used to collect sample for testing, the swab will be considered work product if the swab is consumed for testing.
- 9.8. If a swab or cutting is collected and not consumed during testing, it will be considered evidence and will be returned to the submitting agency. Swabs and cuttings retained with the submitted evidence will be tracked in LIMS as part of the submitted evidence. Swabs and cuttings not retained with the submitted evidence will require tracking in LIMS separate from the submitted evidence. The disposition of the swabs and cuttings will be reported to the customer if a portion of the swab or cutting is preserved for future testing.
- 9.9. Microscope slides generated during testing are considered evidence and will be returned to the submitting agency. Slides retained with the submitted evidence will be tracked in LIMS as part of the submitted evidence. Slides not retained with the submitted evidence will require tracking in LIMS separate from the submitted evidence.
- 9.10. Amplified product is considered work product and not evidence. Amplified products will be destroyed after the case file has been technically approved and reported.
- 9.11. Forensic serological screening of biological material should be performed prior to DNA analysis where applicable.
- 9.12. The following controls and standards shall be used to monitor STR casework:
- 9.12.1. Quantification to estimate the amount of human nuclear and male DNA recovered from extraction shall require the use of appropriate standards.

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- 9.12.2. Amplification shall require the use of a positive and negative control. The positive and negative controls associated with the sample being typed shall be amplified concurrently in the same instrument with the same samples at all loci and with the same primers as the forensic samples. All samples typed shall also have the corresponding amplification controls typed.
- 9.12.3. Extractions will require the use of a reagent blank(s). Reagent blank controls associated with each extraction set being analyzed shall be:
- extracted concurrently.
 - Amplified using the same primers, instrument model, and concentration conditions as required by the sample(s) containing the least amount of DNA.
 - Typed using the same instrument model, injection conditions, and the most sensitive volume conditions of the extract set.
 - If multiple reagent blanks are extracted and quantified within an extraction set, at least one reagent blank shall be amplified and typed. The reagent blank that demonstrates the greatest signal, if any, shall be the reagent blank chosen to amplify and type.
- 9.12.4. STR typing shall use allelic ladders and internal size standards.
- 9.12.5. Peak Analysis and Data Processing is monitored by the human DNA control (positive control) values.
- 9.13. Each laboratory shall check its DNA procedures annually against an appropriate and available NIST standard reference material or standard traceable to a NIST standard or whenever substantial changes are made to the protocol(s).

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- 9.14. The laboratory shall have and follow written guidelines for the interpretation of data including mixture interpretation addressing:
- Major and minor contributors, inclusions and exclusions, and reporting of results and statistics.
 - Inclusions and exclusions
 - Reporting of results and statistics
- 9.15. All procedures must protect against sample contamination. In the event of cross-contamination of a sample from another DNA source during any stage of the DNA typing process, the DNA Technical Leader must be notified. Documentation of the incident, type of contamination, exhibit(s) affected, and all subsequent actions taken must be placed in the affected case file(s). A contamination log must be maintained by the laboratory. The DNA Technical Leader shall determine if any corrective actions are warranted.
- 9.16. A validated procedure must be used for estimating the quantity of DNA recovered from the specimens.
- 9.17. Reagents and Supplies
- 9.17.1. Chemicals, reagents, and supplies shall be of suitable quality, correctly prepared, and demonstrated to be compatible with the methods employed. Upon receipt of reagents chemicals and supplies, the packing slip, purchase request, and items received will be checked that all are in agreement. A copy of the packing slip initialed and dated by the receiver will be maintained by the unit supervisor. If the item(s) received is found to be incorrect, the reagent/supply will not be put into use and will be returned to the vendor.
- Logs must be maintained on all commercial supplies and kits that have expiration dates. For a commercial reagent not having an expiration date,

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the expiration date will be five years from date of receipt unless specified otherwise in policy.

- The STR DNA protocol shall include reagents, extraction techniques, instrumentation, and controls which are standard for DNA analysis and data interpretation. The procedures and reagents for serological testing are maintained in the Forensic Biology Policy and Procedures Manual.
- Reagents shall be labeled with the identity of the reagent, date of preparation, expiration date (where applicable), initials of the individual preparing the reagent, and, as applicable, storage conditions. Records shall be maintained identifying who made the reagent and the components used in preparation.
- Commercial supplies/kits shall be documented as to when they are received, lot number, and expiration date where appropriate.
- In-house prepared reagents will be discarded after one year unless noted otherwise in policy.
- All critical reagents shall be evaluated prior to use in casework. The routine use of controls will be used to ensure the continued reliability of reagents.
- A current inventory of supplies and materials should be maintained.
- Dedicated materials shall be readily identifiable as such.
- All chemicals must be stored, used, and disposed of in a manner conforming to established safety requirements.
- There shall be a file of Safety Data Sheets (SDS's) received from the manufacturer for all chemicals used in the laboratory. These data sheets should be readily available to all laboratory personnel.



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9.18. Quality Control of Reagents and Materials:

- Reagents and materials will be checked against controls known to give acceptable results. If a reagent and/or material yields an expected result as indicated in the appropriate procedure manual, the analyst performing the QC check indicates acceptance by initialing and dating the label and recording in the appropriate reagent logbook. The routine recorded use of appropriate controls during casework is also a suitable method to ensure the reliability of reagents. If a reagent and/or material fails to produce expected results, it must be discarded, re-made, and re-tested. If the reagent and/or material again fails to produce expected results, re-test it against new controls. This rejection of quality control data must also be documented in the reagent preparation logbook.

9.19. Quality Control of Critical Reagents:

- **EZ1 DNA Investigator Kit Components:**
Extract a NIST traceable blood sample or a proficiency test blood sample (with known results) and a reagent blank using the new Qiagen EZ1 DNA Investigator Kit reagent cartridges. Extract the sample and reagent blank using the nuclear DNA pretreatment protocol. Purify the sample and blank using the EZ1 or EZ1-XL. Quantify and amplify the sample and reagent blank using one amplification kit. Run the amplified products on a genetic analyzer. Place documentation in the appropriate QC notebook along with the initials of the QC scientist. The new kit components will be marked as passing if the known sample provides an expected profile of sufficient quality and the reagent blank contains no contamination. The rejection of quality control data must also be documented in the appropriate reagent logbook and the manufacturer will be contacted for additional support and kit and/or component replacement. An example form for documentation and approval of the QC check can be found in the appendix section of this manual.

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Note: The components of the kit are independent of one another and may be substituted with another lot of the same component in the event of non-acceptable results. QC will be performed on the new lot component prior to casework analysis. The components include reagent cartridges, G2 buffer, proteinase K, carrier RNA, MTL buffer.

- **Quantifiler Trio Quantification Kit Components:**

Components of a new kit will be considered acceptable for casework if the slope, R^2 , and Y-intercept values for the standard curves of the large autosomal, small autosomal, and Y targets fall within an acceptable range. The R^2 value shall be greater than or equal to 0.98 for each target. The slope shall be between -3.0 and -3.6 for the small autosomal and Y targets. The slope shall be between -3.1 and -3.7 for the large autosomal target. If the Y-intercept for any target varies by more than ± 0.32 from the previous kit, the supervisor and/or technical leader should be notified. A Y-intercept difference greater than ± 0.32 from the previous kit may require the supervisor to notify users of the new kit about the variation between the new kit and the previous kit.

Documentation of acceptance will be kept in the appropriate reagent logbook along with the initials of the QC scientist. The rejection of quality control data must also be documented in the appropriate reagent logbook and the manufacturer will be contacted for additional support and kit and/or component replacement. An example form for documentation and approval of the QC check can be found in the appendix section of this manual.

- **Note:** The components of the kit are independent of one another and may be substituted with another lot of the same component in the event of non-acceptable results. QC will be performed on the new lot components prior to casework analysis. The components include Quantifiler THP Reaction Mix, Quantifiler Trio Primer Mix, Quantifiler THP DNA Standard, and Quantifiler THP Dilution Buffer.

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- **GlobalFiler and Yfiler Plus Amplification Kit**

Components:

Components of a new kit will be considered acceptable for casework if the control, known sample (e.g. NIST-traceable, proficiency), and allelic ladder produce the correct genotypes or haplotypes. Documentation of acceptance will be kept in the appropriate reagent logbook along with the initials of the QC scientist. The rejection of quality control data must also be documented in the appropriate reagent logbook and the manufacturer will be contacted for additional support and kit and/or component replacement. An example form for documentation and approval of the QC check can be found in the appendix section of this manual.

Note: The components of the kit are independent of one another and may be substituted with another lot of the same component in the event of non-acceptable results. QC will be performed on the new lot component prior to casework analysis. The components of a kit include master mix (containing enzyme), primer set, control DNA, and allelic ladder.

- **Sperm Wash Buffer, Non-sperm Extraction Buffer, or Sperm Extraction Buffer (differential extraction buffers):**

QC of the differential extraction buffers shall consist of a reagent blank as well as a mixed sample containing sperm and blood or epithelial cells which shall be extracted, quantified, and amplified. The amplified products shall then be run on a genetic analyzer to obtain DNA profiles.

Note: The sample used for this QC may be a previous proficiency test sample. Alternatively, the mixed sample may be prepared in-house using known semen, blood or buccal swabs. The QC check data will be maintained in the appropriate QC notebook along with the initials of the scientist performing the QC check.

- **TE Buffer:**

QC of TE buffer shall consist of amplifying a known sample and a negative control using one amplification kit. The amplified samples shall be run on a genetic analyzer.



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The amplification tube for the known shall contain both TE and known sample.

- **Stain Extraction Buffer:**

QC of the stain extraction buffer shall consist of the extraction, quantification, amplification, and analysis of a reagent blank as well as a known blood or buccal swab.

Note: The sample used for this QC may be a previous proficiency test sample or a NIST traceable blood or buccal sample. The QC check data will be maintained in the appropriate QC notebook along with the initials of the scientist performing the QC check.

- **PCI and Proteinase K:**

QC of the PCI or Proteinase K reagent shall be accomplished by either following the QC instructions for the Stain Extraction Buffer or Differential Extraction Buffers.

- **0.39M DTT:**

QC of the 0.39M DTT shall be accomplished by following the QC instructions for the Differential Extraction Buffers.

- **1.0M DTT:**

QC of the 1.0M DTT shall consist of a reagent blank as well as a mixed sample containing sperm and blood or epithelial cells which shall be extracted following the EZ1/ EZ1-XL Bio-robot procedures for a differential extraction. The samples shall be quantified and amplified. The amplified products shall then be run on a genetic analyzer to obtain DNA profiles. The sample used for this QC may be a previous proficiency test sample. Alternatively, the mixed sample may be prepared in-house using known semen, blood or buccal swabs. The QC check data will be maintained in the appropriate QC notebook along with the initials of the scientist performing the QC check.

Note: Passing QC for the Differential Extraction Buffers, TE buffer, Stain Extraction Buffer, PCI, Proteinase K, 0.39M DTT, and 1.0M DTT will be based upon the ability to successfully

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obtain correct DNA profiles without contamination. Documentation of QC will be kept in a reagent QC notebook. A reagent that does not pass QC will be discarded. The reagent will be re-prepared and QC checked again. This shall be documented in the QC notebook. An example form for documentation and approval of the QC check can be found in the appendix section of this manual. The QC checks can be documented on other forms.

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10. Equipment Calibration and Maintenance

- 10.1. The laboratory shall use equipment suitable for the methods employed.
- 10.2. Each laboratory will maintain an inventory of equipment and related software which will include the manufacturer, model, serial number, and location.
- 10.3. The manufacturer's operation manual and instructions will be readily available. Manuals and instructions are maintained in the laboratory, office area of the unit, and/or electronically in ensur.
- 10.4. The laboratory shall have and follow a documented program for conducting performance checks and calibration of instruments and equipment.
- 10.5. A record of performance checks will be maintained in the appropriate log book. The following critical instruments or equipment shall require annual performance checks:
 - 10.5.1. Thermometer traceable to national or international standard(s) that is used for conducting performance checks
 - 10.5.2. Balance
 - 10.5.3. Thermal cycler temperature-verification system
 - 10.5.4. Thermal cycler and quantitative PCR
 - 10.5.5. Robotic systems
 - 10.5.6. Genetic analyzers
 - 10.5.7. Mechanical pipettes
- 10.6. New critical instruments and equipment, or critical instruments and equipment that have undergone repair, service or calibration, shall undergo a performance check before use in casework or database analysis.
 - 10.6.1. The following critical equipment shall undergo a performance check following repair, service or calibration in addition to the annual performance checks:



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- 10.6.1.1. Thermal cycler and quantitative PCR
- 10.6.1.2. Robotic systems e.g. extraction robots
- 10.6.1.3. Genetic analyzers

10.7. The performance check of a thermometer used for performance checks may be accomplished through: (1) certification by an outside vendor; or (2) in-house by the comparison of one or more temperature readings at various time intervals against another NIST-traceable thermometer. Thermometers used for performance checks shall be discarded or re-certified if reading $\pm 1^{\circ}\text{C}$ from the NIST traceable thermometer. Thermometers used to check dry baths shall be discarded or re-certified if reading $\pm 2^{\circ}\text{C}$ from the NIST traceable thermometer. Thermometers used to check refrigerators shall be discarded or re-certified if reading $\pm 5^{\circ}\text{C}$ from the NIST traceable thermometer. Thermometers used to check freezers shall be discarded or re-certified if reading $\pm 5^{\circ}\text{C}$ from the NIST traceable thermometer. Refer to the appendix section of this manual for an example form for documenting the performance check of a thermometer.

10.8. The performance check of a balance may be accomplished either through an outside vendor or performed in-house using certified weights. A record of the performance check will be maintained. Monthly internal calibration checks will be performed using certified weights. The balance will be checked with the 10mg, 100mg, 1g, 10g, and 100g weights. The pass fail range for the 10mg and 100mg is $\pm 1\text{mg}$ and for the 1g, 10g, and 100g is $\pm 0.1\text{g}$. If the balance is not working properly, a "DO NOT USE" sign will be placed on the balance and the supervisor will be notified to determine the appropriate corrective action.

10.9. The performance check of the Driftcon thermal cycler temperature-verification system may be accomplished through certification by an authorized vendor. The vendor must be accredited to the current ISO 17025 standards. After certification from an outside vendor, the certification report from the vendor will be kept in the QC log book. The Driftcon will be shipped via a courier service such as FedEx or UPS in the plastic protective case in which it is stored.

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- 10.10. The performance check of a 9700 thermal cycler includes the system's rate and cycle tests and the use of the Driftcon temperature verification system. This performance check will be conducted quarterly. The recommended months to space out the performance checks are March, June, September, and December. If the test(s) fail, a "DO NOT USE" sign will be placed on the thermal cycler and the supervisor notified to determine the appropriate corrective action.
- 10.11. The performance check of a Veriti thermal cycler includes the system's cycle performance test and the use of the Driftcon temperature verification system. This performance check will be conducted quarterly. The recommended months to space out the performance checks are March, June, September, and December. If the test(s) fail, a "DO NOT USE" sign will be placed on the thermal cycler and the supervisor notified to determine the appropriate corrective action.
- 10.12. The performance check of a 7500 quantitative PCR instrument includes the system's background run, quick systems test, system hardware (function) test, and the use of the Driftcon temperature verification system. Wells showing high fluorescence will be cleaned with de-ionized water and 95% ethanol. If the test(s) fail, a "DO NOT USE" sign will be placed on the thermal cycler and the supervisor notified to determine the appropriate corrective action.
- 10.13. The performance check of a Qiagen EZ1 Biorobot shall be accomplished by extracting a NIST traceable or proficiency test sample and reagent blank. The sample and reagent blank will be processed through quantification, amplification, and typing on a genetic analyzer. A passing performance check will be obtaining correct DNA profiles with no contamination. If the performance check fails, a "DO NOT USE" sign will be placed on the instrument and the supervisor notified to determine the appropriate corrective action.
- 10.14. The performance check of a genetic analyzer will be accomplished by analyzing an amplification positive and negative and a ladder. A positive, negative, and ladder should be injected into each capillary. A passing performance check will

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be to obtain correctly typed profiles with no contamination. If the performance check fails, a “DO NOT USE” sign will be placed on the instrument and the supervisor notified to determine the appropriate corrective action.

- 10.15. The performance check of a mechanical pipette will be accomplished by certification by an outside vendor. If the pipette is not working properly, the pipette will be repaired by the vendor or the pipette will be disposed. Calibration of pipettes will be documented by a sticker on the pipette bearing the technician’s initials/date and/or a log sheet of calibrated pipettes retained in the equipment log book. If a pipette appears to be out of calibration or broken, the pipette will be sent to an authorized vendor for repair. Pipettes should be cleaned with ethanol or isopropyl alcohol as needed and prior to sending for calibration or repair.
- 10.16. Laboratory weights shall be externally calibrated at least once every three years. The weights shall be packaged individually using a protective case or other protective material such as foam or bubble wrap to prevent damage during shipping.
- 10.17. The laboratory shall have a schedule and follow a documented program to ensure that instruments and equipment are properly maintained. The laboratory shall retain documentation of maintenance, service or calibration. Permanent logs of calibration, maintenance, and repairs will be maintained next to individual instruments or another designated location in the lab. Dedicated equipment will be readily identifiable and tagged.
- 10.17.1. The temperatures of dry baths, heat blocks, refrigerators, freezers, and incubators will be recorded weekly. If the temperatures are not in range, the thermostat may be adjusted and the temperature checked again after one hour. Repeat if necessary. If the equipment is found to not be working properly, a note will be placed on the equipment removing it from service and the supervisor notified.
- 10.17.1.1. The range for a dry bath/heat block set at 37°C, 56°C, or 95°C is $\pm 1^\circ\text{C}$.

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10.17.1.2. The range for a refrigerator set at 5°C is $\pm 3^{\circ}\text{C}$.

10.17.1.3. The range for a freezer set at -20°C is $\pm 5^{\circ}\text{C}$.

10.17.1.4. The range for an incubator set at 37°C is set at $\pm 1^{\circ}\text{C}$.

10.17.2. On a monthly basis, balances will have an internal calibration check using certified weights. The balance will be checked with the 10mg, 100mg, 1g, 10g, and 100g weights. The pass/fail range for the 10mg and 100mg is $\pm 1\text{mg}$ and for the 1g, 10g, and 100g is $\pm 0.1\text{g}$. If the balance is not working properly, a "DO NOT USE" sign will be placed on the balance and the supervisor will be notified to determine the appropriate corrective action.

10.17.3. The maintenance plan for an EZ1 or EZ1-XL Bio-robot will consist of daily, monthly, and annual maintenance.

10.17.3.1. Daily (after each run):

- Clean the piercing unit with 70% isopropyl alcohol
- Check that the tray is clean. If necessary clean with 70% isopropyl alcohol.
- Wipe the O-rings of the tip adapters, if dirty, with a tissue.

10.17.3.2. Monthly:

- Perform the daily maintenance
- Grease the tip adapters by applying a small amount of silicon grease to the surface of the O-rings using a filter tip. Place the tip on the plunger and rotate to distribute the silicon grease evenly. This will ensure good contact between tip adapters and filter tips to prevent leaking from the tips.

10.17.3.3. Annual:

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- Perform the daily maintenance
- Perform the monthly maintenance
- Perform the temperature and pipetting accuracy tests. Refer to the appendix section of this manual for temperature and pipetting accuracy test instructions.
Note: A performance check must be performed after completion of the annual maintenance prior to casework use.

10.17.4. The maintenance plan for a real time PCR 7500 will consist of daily, monthly, six (6) months, and annual maintenance for use with the HID Real-Time PCR Analysis Software and Quantifiler Trio. The 6 months maintenance should be performed upon expiration of the previous dye calibrations, which is approximately 6 months; however, these calibrations may be done earlier and as needed. The manufacturer refers to the 6 months maintenance as semiannual, but the maintenance should be performed by or during the sixth (6th) month from the previous maintenance.

10.17.4.1. Daily or as needed:

- Check and replace the bulb and or fuse as needed. Bulb replacement requires a background run to be completed.

10.17.4.2. Monthly:

- Perform a background run
- Run the Quick Systems Test
- Thermal cycler maintenance (clean wells showing high fluorescence using DI water and 95% ethanol.)
- Run the System Hardware (Function) Test

10.17.4.3. Six (6) Months

- ROI Calibration
- Background Calibration



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- Optical Calibration
- Dye Calibrations

10.17.4.4. Annual:

- Calibration and maintenance by an outside vendor
- Following annual maintenance, a performance check must be performed prior to casework use.

10.17.5. The maintenance for a 9700 or a Veriti thermal cycler will consist of monthly maintenance and quarterly performance checks.

10.17.5.1. Monthly:

- Clean the wells and sample cover using 95% ethanol or isopropyl alcohol.

10.17.5.2. Quarterly:

- 9700: Perform the Rate and Cycle Tests
- Veriti: Perform the Cycle Performance Test
- Run the Driftcon Verification System for both the 9700 and Veriti
- If any test(s) fail, a "DO NOT USE" sign will be placed on the instrument and the supervisor will be notified to address the appropriate repair.

10.17.6. The maintenance for a 3500 genetic analyzer will consist of daily (before use), monthly and annual maintenance.

10.17.6.1. Daily / before use (3500)

- Check and replace polymer that is over two weeks old or as needed
- Check and replace buffers and water as needed
- Replace septa when replacing buffer or water
- Check for bubbles in the pump block and channels

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- Check for leaks and dried residue
- Clean as needed
- Restart the computer and instrument as needed

10.17.6.2.Monthly (3500):

- Perform back-up and archive of runs
- Defragment the C and D hard drives
- Purge plates from the Library
- Run the Wash Pump and Channels wizard
- Flush the pump trap and empty the water trap waste container
- Restart the instrument and computer

10.17.6.3.Annual (3500)

- Annual maintenance and calibration will be performed by an outside vendor
- A performance check must be run following the annual maintenance prior to use in casework. If the genetic analyzer is not functioning properly, a “DO NOT USE” sign will be placed on the instrument and the supervisor notified to determine the repair
- It is recommended to replace the back-up jump drive annually.

10.17.7. The maintenance for a microscope will consist of annual maintenance.

10.17.7.1.Annual:

- Perform Kohler illumination
- Clean optics using lens paper
- Clean the body with 70% isopropyl alcohol
- If a microscope is not functioning properly, remove the microscope from service and notify the supervisor to determine the correct repair.

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- The annual calibration may be documented on the microscope body with a sticker.

10.17.8. The pH meter will be calibrated upon each use using commercially purchased buffers. The pH meter will be calibrated using pH 4.0, 7.0, and 10.0 buffers. The electrode will be stored in pH 7.0 buffer and may be refilled with KCl as needed. If the pH meter is not functioning correctly, a “DO NOT USE” sign will be placed on the meter and the supervisor notified to determine the correct repair.

10.17.9. Fume hoods and bio-hoods will be externally calibrated annually. If a hood is found to not be functioning correctly, a “DO NOT USE” sign will be placed on the hood and the supervisor notified to initiate a repair call or e-mail to building maintenance.

10.18. The laboratory shall document all critical equipment and instruments. Documentation must include the schedules for and records of all repairs, service, or calibrations for the critical equipment and instruments. Critical equipment or instruments are those requiring calibration prior to use and periodically thereafter when the accurate calibration of that instrument directly affects the results of the analysis.

10.19. Critical instruments or equipment that are not listed in Standard 10.6.1 are not required to have a performance check after repair, service, or calibration.

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11. Reports

There must be policies and checks and balances in place to ensure the reliability and completeness of the documentation, data analysis, reports, and review process.

11.1. Casework Documentation

11.1.1. Documentation must be in such a form that a competent analyst or supervisor or technical leader, in the absence of the primary analyst, would be able to evaluate and to interpret the data.

11.1.2. Documentation must include, but is not limited to, data obtained through the analytical process. It should also include information regarding the packaging of the evidence upon receipt and the condition of the evidence itself, with particular attention to those factors that are relevant to the preservation of the biological material. The start and stop date of testing must be documented in the case record.

11.1.3. All documentation of procedures, standards and controls used, observations made, results of the tests performed, photographs, electropherograms, communications, etc., which are used to support the analyst's conclusions, must be preserved as a record according to laboratory policy. Results will be preserved by photography, computer disks, electropherograms, or other suitable means.

11.1.4. Statistical Evaluation

The frequency of occurrence for a questioned DNA profile is calculated using a scientifically valid method from an established population database.

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11.2. Report Writing

In accordance with the FBI QAS, reports with DNA results will contain at a minimum the following information:

- Case number
- Description of evidence
- Report date
- The DNA loci evaluated
- Description of technology
- Results
- Conclusions
- Disposition of evidence
- An interpretative statement (either quantitative or qualitative to support all inclusions)
- Signature (or apply a computer-generated signature) and title of the reporting analyst (Administrative Review).

11.3. Reports will indicate that no examination was performed when items are submitted that have no testing performed.

11.4. Reports will indicate when items have been collected or created and are being preserved for future testing.

11.5. Reports will contain the results of both completed and partial testing.

11.6. Reports will provide the reason(s) when results are inconclusive.

11.7. Reporting of initial casework profile entries into the CODIS database is required. The results of the initial search of the CODIS database will be reported except for solved cases with no case to case association(s). Reporting of an association resulting from a CODIS database search is required. Refer to the CODIS Manual for additional information.

11.8. Reports will clearly identify when results are from an external provider.



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11.9. Except as otherwise provided by state or federal law, reports, case files, DNA records and databases shall be confidential.

11.9.1. The laboratory shall have and follow written procedures to ensure the privacy of the reports, case files, DNA records and databases.

11.9.2. The laboratory shall have and follow written procedures for the release of reports, case files, DNA records and databases in accordance with applicable state or federal law.

11.9.3. Personally identifiable information shall only be released in accordance with applicable state and federal law.

11.9.4. The reports and records of the Forensic Biology and CODIS section will be maintained in the central and regional laboratories. Lab reports, case files, DNA records, and databases are confidential relative to TCA 10-7-504(a) confidential records- (a). The information contained in these records are not open to inspection by members of the public. The information in these records shall be disclosed to the public only in compliance with a subpoena or an order of a court record. These records will be open to inspection by elected member of the General Assembly if such inspection is directed by a duly adopted resolution of either House or of a standing or joint committee of either House. A copy of the lab report will be made available to the submitting agency and to the appropriate District Attorney General.

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12. Review

12.1. Data, documentation, statistical evaluation, and conclusions must be reviewed independently by a second qualified analyst who has been competency tested in the task(s) that the review encompasses. The technical review will be documented prior to uploading or searching in SDIS or issuing a report. Both individuals must agree on the interpretation of the data and the conclusions derived from that data. Scientists cannot technically review their own work.

12.1.1. An individual conducting technical reviews shall be an analyst or technical reviewer qualified in the method, technology, typing test kit, platform, and interpretation software being reviewed having completed a competency test prior to participating in the technical review of DNA data. Each technical reviewer will participate in an external proficiency testing program on the same technology, platform and typing amplification test kit used to generate the DNA data being reviewed.

12.2. The following elements shall be checked during a technical review and the review documented on the first page of the case notes with the reviewer's initials and date of review.

12.2.1. A review of all case notes, worksheets, and electronic data or printed electropherograms/images that support the conclusions for conformance with proper procedures and policy. This review includes verifying calculations and data transfers.

12.2.2. A review that all screening results are accurately reported and proper controls were used for each screening test performed.

12.2.3. A review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms or images).

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- 12.2.4. A review of all profiles to verify correct inclusions and exclusions as well as a review of any inconclusive results for compliance with laboratory guidelines.
- 12.2.5. A review of all controls, internal lane standards, and allelic ladders to verify that the expected results were obtained.
- 12.2.6. A review of statistical analysis, if applicable. Associations are supported qualitatively or with statistical weight. Statistics shall be reported for probative associations.
- 12.2.7. A review of the final report to verify that the results/conclusions are supported by the data and that the report addresses each tested item or its probative fraction.
- 12.2.8. Verify the eligibility of CODIS entries. Verify that all profiles entered into CODIS are eligible and have the correct DNA types and correct specimen category. CODIS entries will be compared directly to the electropherogram when verifying that correct types have been entered.
- 12.2.9. Verify that prior to upload to or search of SDIS, that the following have been verified for DNA profiles:
- Eligibility for CODIS
 - Correct DNA types
 - Appropriate specimen category
- 12.2.10. Verify that prior to entry of a DNA profile into a searchable category of SDIS, the following criteria were verified by two concordant assessments by a qualified analyst or technical reviewer:
- Eligibility for CODIS
 - Correct DNA types
 - Appropriate specimen category

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12.3. The administrative review shall be conducted by an individual who is not the author of the report. Cases requiring CODIS entry verification will be administratively reviewed by a current or previously qualified analyst. The review 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 errors including spelling and grammatical accuracy and that information specified in section 11.2 is present and accurate. CODIS entries will be checked against the electropherogram for clerical errors.

12.3.2. A review of all administrative and examination records to ensure that the records are uniquely identified with lab number and initials.

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

12.3.4. The completion of the administrative review is documented on the first page of the case notes with the reviewer's initials and date of review.

12.4. It is the responsibility of the supervisor and technical leader to assure that all deficiencies are acknowledged and that any corrective action is successfully completed. In the event of an unresolved disagreement between the technical reviewer and the analyst, the casework is to be referred to the Forensic Biology Supervisor and the DNA Technical Leader. If the disagreement is still unresolved, the matter must be referred to the Assistant Director who may confer with the Quality Assurance Manager.

12.5. The laboratory shall have and follow a documented procedure for the verification and resolution of database matches (See CODIS Manual).

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- 12.6. The laboratory shall have and follow a program that documents the annual monitoring of the testimony of each analyst (Refer to the division Quality Assurance Manual)



13. Proficiency Testing

Proficiency testing is used to demonstrate the quality performance of the laboratory and its analysts. This is accomplished by the analysis and reporting of results from appropriate biological specimens submitted to the laboratory as open case evidence. The laboratory will use an external proficiency test provider that is in compliance with the current International Organization for Standards.

13.1. Open Proficiency Testing

Open proficiency test specimens are presented to the laboratory and its analysts as proficiency specimens and are used to demonstrate the analytical and interpretive capability of each analyst and the laboratory's methods, equipment, and materials. Two (2) external proficiency tests are given each year to each scientist performing screening and to each analyst performing screening and DNA analysis. A DNA technical reviewer shall undergo semiannual proficiency testing in each technology performed to the full extent in which they participate in casework. Beginning January 1, 2014, a scientist performing only body fluid identification will be required to successfully complete two external proficiency tests per calendar year.

13.2. Frequency

Semi-annual is used to describe an event that takes 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 two events is at least four months and not more than eight months. Such 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. The proficiency cycle is defined as the due date of the proficiency test provider.

13.3. Analysis

All open proficiency test specimens must be analyzed and interpreted according to the protocols approved for use at the time of the open proficiency test. The materials and kits used

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should reflect those currently in use by the forensic biology laboratory.

13.4. Documentation

At a minimum, the following proficiency test data and information must be retained:

- Proficiency test set identifier
- Identity of the analyst
- Date of analysis and completion
- Copies of all data and notes supporting the conclusions
- Quantification data sheets
- Original or duplicate electropherograms as appropriate
- Analyst's results and conclusions
- The proficiency test results
- Any discrepancies noted
- Corrective action taken

Note: Analyst information and result sheets must be submitted to the QA Manager.

13.5. Review and Reporting of Proficiency Test Results

The DNA Technical Leader shall review all test materials in a timely manner. All original notes, electropherograms, records, and other data pertaining to the proficiency test results will be retained by the DNA analyst and/or supervisor. Technical reviews shall be performed on proficiency tests by an analyst who has completed his or her own proficiency test prior to the review.

13.6. Evaluation of Proficiency Tests

At a minimum, the following criteria are to be used for evaluation of the proficiency tests:

- Are all reported screening results correct or incorrect?
- Are all reported inclusions correct or incorrect?
- Are all reported exclusions correct or incorrect?
- Are all reported genotypes correct or incorrect according to consensus genotypes or within the laboratory's interpretation guidelines?
- Are all results reported as inconclusive or uninterpretable consistent with written laboratory guidelines? The basis for

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inconclusive interpretations in proficiency tests must be documented.

- All discrepancies/errors and subsequent corrective actions must be documented.
- All final reports are graded as satisfactory or unsatisfactory.
- A satisfactory grade is attained when there are no errors in screening and no analytical errors for the DNA profile typing data. Administrative errors shall be documented and corrective actions taken to minimize the error in the future.
- All proficiency test participants shall be informed of the final test results. The DNA Technical Leader shall also have a documented notification of the final results of all participants. If applicable, the Technical Leader will inform the CODIS Administrator of all non-administrative discrepancies that affect the typing results and/or conclusions at the time of discovery.

- 13.7. Individuals routinely utilizing both manual and automated methods shall be proficiency tested in each at least once per year to the full extent in which they participate in casework.
- 13.8. For individuals qualified in multiple technologies (e.g. STR and Y-STR testing), each such individual must be externally proficiency-tested in each technology semiannually. All applicable samples in a single proficiency test shall be worked for each technology.
- 13.9. Newly qualified individuals shall enter the external proficiency testing program within six months of the date of their qualification.
- 13.10. Qualified analysts who have recently completed competency testing on a new or revised DNA procedure, must incorporate that method in the next cycle of proficiency testing.
- 13.11. Each analyst shall be assigned and complete his/her own external proficiency test.
- 13.12. Typing of all CODIS core loci shall be attempted for each technology performed.

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14. Corrective Action

14.1. The laboratory shall establish and follow a corrective action plan to address when discrepancies are detected in proficiency tests, casework/database analysis, testimony, and audits. A laboratory corrective action plan shall define what level/type of discrepancies are applicable to this practice and identify (when possible) the cause/effect of the discrepancy, corrective actions taken, and preventive measures taken (where applicable) to minimize its reoccurrence. Refer to the Division Quality Assurance manual for further information.

14.2. Corrective actions shall not be implemented without the documented approval of the technical leader.

14.2.1. Analytical/Technical Errors

Any significant discrepancy in the proficiency test results determined to be the consequence of an analytical, technical, or methodological error shall result in suspension of the analyst's casework until the cause of the problem is identified and corrected. The QA Manager or DNA Technical Leader will determine the need to audit prior casework of the involved individual(s). Any discrepancy in a proficiency test determined to be the result of administrative error (clerical, sample confusion, improper storage, documentation, etc.) will be corrected according to laboratory policy. Once the cause of the discrepancy has been identified and corrected, the affected analyst must successfully demonstrate that the appropriate corrective action has taken place so as to minimize the recurrence of the discrepancy. If appropriate, an additional open proficiency test may be required from the individual(s) involved.

14.2.2. Systematic Error

Any significant discrepancy in a proficiency test determined to be the result of systematic error (equipment, materials, and environment) may require

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an immediate review of all casework since the DNA laboratory's last successfully completed proficiency test. All DNA analysts shall be made aware of the error and the corrective action.

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15. Audits

- 15.1. The laboratory shall be audited annually in accordance with the FBI Quality Assurance Standards. The annual audits shall occur every calendar year and shall be at least 6 months and no more than 18 months apart. Audits shall be conducted by an audit team comprised of qualified auditor(s) having at least one team member who is or has been an analyst previously qualified in the laboratory's current DNA technologies and platform.
- 15.2. At least once every two years, an external audit shall be conducted by an audit team comprised of qualified auditor(s) from a second agency (ies) and having at least one team member who is or has been an analyst previously qualified in the laboratory's current DNA technologies and platform.
- 15.2.1. Each analyst, casework CODIS administrator, and technical leader shall have his/her education, experience and training qualifications evaluated and approved during two successive, separate external audits conducted after July 1, 2004. Approval of an individual's education, experience and 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. For internal audits, the auditor or audit team shall have the following expertise: currently qualified auditor and currently or previously qualified as an analyst in the laboratory's current DNA technologies and platform.
- 15.4. Internal and external audits shall be conducted utilizing the most current version of the FBI DNA Quality Assurance Standards Audit Document.

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

15.5.1. External audit documentation and laboratory responses shall be provided to the FBI within 30 days of laboratory receipt of the audit documents or report.

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

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16. Safety

- 16.1. The laboratory shall have and follow a documented environmental health and safety program. This program shall include the following:
- 16.1.1. A blood borne pathogen and chemical hygiene plan.
 - 16.1.2. Documented training on the blood borne pathogen and chemical hygiene plan.
 - 16.1.3. The DNA laboratory shall operate in accordance with regulations of the pertinent federal, state, and local health and safety authorities.
 - 16.1.4. Refer to the TBI Occupational Safety Manual and the Occupational Safety and Health Program Plan on the TBI intranet for further details on the health and safety program.
- 16.2. The laboratory's environmental health and safety program shall be reviewed once each calendar year and such review shall be documented.

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17. Outsourcing

17.1. When using a vendor laboratory, the vendor laboratory performing forensic DNA analysis shall comply with the current version of the FBI Quality Assurance Standards and the accreditation requirements of federal law.

17.1.1. The laboratory that outsources DNA sample(s) to a vendor laboratory to generate DNA data that will be entered into or searched in CODIS shall require the vendor laboratory to provide documentation of compliance with these Quality Assurance Standards and the accreditation requirements of federal law. The laboratory shall maintain such documentation.

17.2. The technical leader shall document approval of the technical specifications of the outsourcing agreement with a vendor laboratory before it is awarded. Such documentation shall be maintained by the laboratory.

17.3. The TBI Forensic Biology laboratory shall not upload or accept DNA data for upload to CODIS from any vendor laboratory or agency without the documented prior approval of the technical specifications of the outsourcing agreement and/or documented approval of acceptance of ownership of the DNA data by the technical leader.

17.4. The laboratory shall have and follow a procedure to verify the integrity of the DNA data received through the performance of the technical review of DNA data from a vendor laboratory.

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

17.6. Prior to the upload of DNA data to SDIS or the reporting of search results, the technical review of a vendor laboratory's DNA data shall be performed by an analyst or technical reviewer who is qualified or previously qualified in the

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technology, platform and typing amplification test kit used to generate the data and participates in the proficiency testing program. Vendor lab data not requiring an SDIS upload/search or reporting/interpretation of the data by the TBI does not require a technical review. The lab may issue a report or cover letter referring to the vendor lab report for results with a statement indicating there is no CODIS eligible profile.

- 17.7. The technical review for outsourced cases shall include the following elements:
 - 17.7.1. A review of all DNA types to verify that they are supported by the raw and/or analyzed data (electropherograms or images).
 - 17.7.2. A review of all associated controls, internal lane standards and allelic ladders to verify that the expected results were obtained.
 - 17.7.3. A review of the final report (if provided) to verify that the results/conclusions are supported by the data. The report shall address each tested item (or its probative fractions) submitted to the vendor laboratory.
 - 17.7.4. Verification of the DNA types, eligibility, and the correct specimen category for entry into CODIS. Allele calls should be based on the vendor lab guidelines (e.g. stochastic and analytical thresholds).
 - 17.7.5. Completion of technical reviews of vendor lab DNA data will be documented in the case file with the reviewer's initials and date. The review may be documented on an outsourced STR casework review checklist form. An example form is in the appendix section of this manual. This form may be used as the first page of the TBI case notes, if applicable, to document the technical and administrative reviews.
- 17.8. The laboratory outsourcing DNA sample(s) to a vendor laboratory or accepting ownership of DNA data from a vendor laboratory shall have and follow a procedure to perform an on-

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site visit(s) of the vendor laboratory, provided however, that an on-site visit shall not be required when only technical review services are being provided. An on-site visit shall include, at a minimum, the following elements:

- 17.8.1. A documented initial on-site visit prior to the vendor laboratory's beginning of casework analysis for the laboratory.
- 17.8.2. The on-site visit shall be performed by the technical leader or a designated employee of the laboratory who is a qualified or previously qualified DNA analyst in the technology, platform, and typing amplification test kit used to generate the DNA data. Alternatively, the technical leader may accept an on-site visit conducted by a designated FBI employee.
- 17.8.3. If the outsourcing agreement extends beyond one year, an annual on-site visit shall be required.
- 17.8.4. The laboratory may accept an on-site visit conducted by the FBI or another NDIS-participating laboratory using the same technology, platform, and typing amplification test kit used for the generation of the DNA data, or coordinated by a designated FBI employee, and the technical leader shall document the review and approval of such on-site visit.



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APPENDIX A

Organization of Worksheets in the Forensic Biology Units

1. The first page of the examination records packet shall document the start and stop date for testing, the total number of pages in the packet, and the official technical and administrative review dates of the packet. The technical and administrative reviewer(s) will initial and date the completion of the reviews.
2. If the Request for Examination form only asks for conventional serology (i.e. identification of sperm / semen, blood, etc.) and a report is issued, this is considered a packet of information and case notes (worksheets, etc.) will be sequentially numbered.
3. If additional evidence is received on a case, the new packet of completed worksheets will be numbered sequentially for that particular packet. For example: A blood standard has been submitted and the worksheets pertaining to that submission are numbered "1" and "2" with the total number of pages being "2" (this is only if DNA analysis was not performed and a report was issued). If DNA analysis was performed before the report was issued, the packet is grouped as follows: Serology worksheets / notes first, followed by DNA analysis worksheets / electropherograms, and any statistics sheets last.
4. DNA analysis case notes (worksheets, etc.) will be arranged by run dates. For example: If proficiency test 11-574 Item # 1 through # 4 were run on 08/10/11, all worksheets [DNA extraction through the allele sheet(s)] will be grouped together. If Item # 2 was rerun on another date, this would be designated "run # 2" and all worksheets for that particular run would be grouped together. If there are multiple runs, the allele call sheets or a summary sheet that contains all the allele calls may be added to the end of all electropherograms (placed before any statistics sheet(s)).
5. Administrative Documentation and Examination Documentation:
 - A. Administrative Documentation (page numbers are not required) must have lab case number and should also have analyst's initials. Administrative documents belonging to a multi-section master folder may not have the analyst's initials.

These may include, but are not limited to: phone log, TBI Request for Examination forms, victim sexual assault exam / history information sheet(s), District Attorney



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DNA correspondence, Property / Evidence Report, and CODIS Specimen Detail Match, and Match Estimation reports / documents.

These items should be stapled to the **left** inside of the case folder of a single-section case (normally the Evidence Receiving unit will staple the original Request for Examination forms to the left inside of the master folder). If it is a multi-section case, initialed copies or originals of these administrative documents may be placed before the examination documentation but are not numbered. It may be necessary for the analyst to use a two-hole punch, heavy duty staples, multiple folders, etc. to make sure the casework is secured in the case file.

- B. Examination (Technical) Documentation (excluding official reports), lab case number and exhibit number, if applicable, examiner's hand-written initials, and page numbers are required on each page. These sheets are on the **right** inside of the folder and should be placed in numerical exhibit order within the examination documentation.

Conventional serology worksheets only:

- Administrative documents (if multi-section)
- Case summary sheet (if applicable, this page is optional)
- Conventional serology worksheets. Example serology worksheets can be found on the document control system. The notes shall include the following: description of the packaging and contents, type of test performed, date of testing, results of testing, results of controls, and documentation of any swabs or cutting taken for future DNA. Photos and/or drawings should be used to indicate areas tested on larger items of evidence. Photos must have the lab number, analyst's hand-written initials, and date of photograph(s).

Conventional serology and DNA worksheets:

- Administrative documents (if multi-section)
- Case summary sheet (if applicable, this page is optional)
- Conventional serology worksheets.

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- DNA worksheets. Example DNA worksheets can be found on the document control system. For DNA documentation, individual samples on worksheets and electropherograms must be identified with the laboratory and exhibit number. A sample number may be used on DNA worksheets along with the laboratory and exhibit number for the purpose of tracking.
- The DNA documentation should be placed in the order below and will contain the information associated with each sheet.
 - STR Sample sheet - contains the exhibit number and evidence description of the items being processed.
 - Reagent QC sheet – the reagents used for extraction, quantification, amplification, and electrophoresis shall be documented with the expiration date, and lot number (if applicable). The date of testing shall be documented. The instrument(s) used for analysis shall be identified for each step. This sheet may be placed before the DNA extraction sheet.
 - DNA Extraction sheet – sample number (if applicable), exhibit number, date and time of extraction, type of extraction performed (if robotic, the protocol will be specified as well), the final elution volume of the sample.
 - Quantification set-up sheet (if applicable) – the location of standards and samples loaded onto the optical plate will be documented.
 - Standard Curves (Quantifiler Trio only)
 - Quantification Data sheet – The following columns will be printed: the well, sample name, target, Ct, male: female ratio, degradation index and quantity.
 - Amplification Calculation sheet – This sheet shall contain the sample number (if applicable), exhibit number, date of amplification, concentration of sample, the volumes of TE and DNA used for amplification.

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- 3500 Instrument Set-up sheet (if applicable) – This sheet will indicate the location of samples loaded into the plate.
 - Electropherograms should be placed in the order of negative control, positive control, allelic ladder(s) and samples. If applicable, a mixture worksheet or interpretation notes should be placed before or after the electropherogram being interpreted.
Electropherograms used for interpretation must be printed and placed in the case file. Electropherograms that are not printed will not be used for interpretation. If an electropherogram in the case file is not used, it must be documented in the case file. The scientist will initial, date, and provide a reason why the electropherogram is not being used.
 - STR Allele Result sheet (summary allele sheet, if applicable). This sheet contains alleles at or above the analytical threshold for samples and controls run with GlobalFiler or Yfiler Plus.
 - PopStats Sheet (if applicable) – This printed sheet will reference the database used and the Specimen ID (laboratory number and exhibit number).
 - Y-STR database search statistics (if applicable)- This sheet is printed from the web page.
 - CODIS Entry Data sheet (keep in case file folder until technical review has been conducted and approved and then placed in a common file in the CODIS Administrator's office). This sheet may be used by the CODIS unit to document search results. The sheet will be retained with the administrative page upon return from the CODIS unit.
6. The official chain of custody will be maintained electronically. In the event of LIMS shut down, the analyst may revert to a handwritten chain of custody as outlined in the Evidence Receiving Unit's Standard Operating Procedure.
7. Controls are to be utilized and documented on the allele call sheet for each particular run.

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8. Case notes and records of observations are subject to subpoena or discovery and must be of a permanent nature. Therefore, **DO NOT USE WHITEOUT** for any reason. No obliterations, pencil (can be used only for drawing), write-overs, or erasures. Corrections are to be made by an initialed, single line strike-through. Contemporaneous changes, alterations, and additional notations (including interlineations) made in case notes must be initialed by the person making the additions. If changes, additions, or corrections are made to the case file after the stop date, the change, addition, or correction must be initialed and dated by the scientist or person responsible.
9. Outsource Case Files
 - a. Outsource case files containing a CODIS eligible profile should be given a TBI lab number in LIMS when the TBI has taken ownership of the data. Outsource case files without a CODIS eligible profile may be given a TBI lab number in LIMS at the discretion of lab management.
 - b. The case file may be tracked in LIMS, but the case file is not considered evidence and tracking (chain of custody) is not required.
 - c. TBI generated paperwork and outsource paperwork should be attached to opposite sides of the folder.
 - d. A technical review worksheet should be used to document the review. An example worksheet is in Appendix C of this manual. Other worksheets or forms may be used to document the review, but should capture the same information.

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Appendix B

Guide for Casework Review

1. Lab case#, exhibit# (if applicable), and analyst initials are on each page
2. Exhibits and descriptions are consistent with the Request for Examination form
3. Packaging and contents are described in the notes
4. Proper controls were used and provided expected results
5. Dates and results of testing are documented
6. STR cutting dates are documented
7. Location of testing and cuttings/swabbings are documented
8. Results of all screening tests are in the final report
9. Appropriate tests and procedures used
10. Case notes, calculations, worksheets, and electropherograms support conclusions
11. DNA profiles are supported by the electropherograms and/or raw data
12. All controls, internal lane standards, and allelic ladders provided expected results
13. All inclusions and exclusions were reported correctly
14. All inconclusive results are in accordance with policy and a reason is provided in the final report
15. Associations are qualified either qualitatively or with statistics
16. Statistics are calculated for all probative inclusions and are correct
17. Each tested item or probative fraction is addressed in the final report
18. CODIS entries are correct based on direct comparison to electropherograms
19. Profiles for CODIS entry are eligible and are in the correct specimen category
20. Appropriate standards, district attorney requests, and elimination standards have been requested
21. Case file reviewed for clerical errors including grammar and spelling
22. Chain of custody and disposition of evidence are correct
23. CODIS Entries have been entered correctly. Compare directly to the electropherogram.
24. Final report is complete and signed
25. The technical and administrative reviews are documented on page 1 of the case file

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Appendix C

Outsourced STR Casework Review

Page _____

Vendor Lab # _____ TBI Lab # _____

- | | Yes | No | NA |
|--|--------------------------|--------------------------|--------------------------|
| 1. Are there acceptable profiles to be entered into CODIS? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Is the appropriate examination documentation present and complete? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Were the appropriate tests performed? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. Were proper controls used and expected results obtained? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. Were expected results obtained for internal lane standards and allelic ladders? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. Are the DNA profiles supported by the raw and/or analyzed data? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. Are the results and conclusions in the final report supported by the data? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. Were the appropriate statistical calculations provided and correct? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. Does the final report address each tested item or its probative fraction? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Technical Reviewer _____ Date _____

- | | | | |
|--|--------------------------|--------------------------|--------------------------|
| 10. Is the CODIS Data Entry Sheet(s) present? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. Are the profiles entered into CODIS eligible? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 12. Are the profiles entered in the correct CODIS specimen category? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 13. Are the DNA profiles entered into CODIS correctly based on the electropherogram? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 14. Does the TBI report or memo address all the profiles entered into CODIS? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 15. Have appropriate standards and elimination standards been requested? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 16. Is the chain of custody and evidence disposition complete and accurate? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| 17. Is the TBI lab report or memo accurate and complete? | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Technical Reviewer _____ Date _____

Administrative Reviewer _____ Date _____

Total Pages _____

Elements 1-9 address the review of the vendor lab's data. Elements 10-17 address the review of CODIS entries and the TBI Lab report.

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Appendix D

9700 and Veriti Thermal Cycler Maintenance Instructions

I. 9700 Rate and Cycle Tests:

Prior to beginning the Rate or Cycle tests, be sure to place the 96 well Full Plate Cover on the instrument and close the lid.

1. From the opening menu screen on the 9700, press "Util" (F4).
2. Press "Diag" (F1)
3. Press "System" (F2)
4. Press "Rate" (F1) for rate test or "Cycle" (F2) for Cycle test.
5. Press "Cont" (F1) to begin test. *Note:* This window is the same for both Rate and Cycle tests.
6. Once the selected test has completed, record the result [Pass/Fail] in the maintenance log and press "Cancel" (F5) to exit the test window.
7. Once both tests have been completed, press "Exit" (F5) on each successive screen to back out to the opening 9700 menu screen.
8. If either of the tests fails, repeat the failed test. If the test fails again contact the supervisor and place a "NOT IN USE" sign on the instrument.

II. Veriti Cycle Performance Test

Prior to beginning the cycle performance test, be sure to place a 96 well reaction plate or thermal isolation frame in the instrument and close the lid.

1. In the Main Menu screen, touch Tools Menu.
2. In the Tools Menu screen, touch **Run Cycle Performance Test**.
3. Follow the instructions on the screen to perform the test. The results are displayed at the end of the test.
4. Once the selected test has completed, record the result [Pass/Fail] in the maintenance log.
5. If either of the tests fails, repeat the failed test. If the test fails again contact the supervisor and place a "NOT IN USE" sign on the instrument.

III. Driftcon Temperature Verification System:

1. Turn on the 9700 or Veriti thermal cycler.

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2. For the 9700, Press “User” (F5) and arrow over to “drfcon” to select the Driftcon user settings. Press “Accept” (F1). For the Veriti, select “Driftcon” from the Main Menu touch screen.
3. Open the cover of the 9700 or Veriti and remove any trays present on the heating block.
4. Turn on the computer.
5. Remove the Driftcon probe from the case and place it on the 96 well block. Place the thin cork cover on top of the probe. For the 9700, the top should be closed and latched. For the Veriti, the top should be closed, but caution should be used if latching. Some Veriti tops can apply to much pressure to the Driftcon probes causing probe failure. If this results, the top may be closed and held down by hand in lieu of latching.
6. Connect one end of the data cable to the Driftcon Probe and the other end to the hardware module.
7. Connect the USB cable to the back of the hardware module and to the computer. You should see a green light begin blinking on the hardware module.
8. Double click on the “Driftcon” shortcut icon on the desktop to launch the software.
9. Click on the profile icon (e.g. Mr A Admin) and type in the password *driftcon*. If you have a user profile established, log in under that profile.
10. Click on the “Start” button to begin the *Start Measurement Wizard*.
11. On page 1, visually check that *PCR Instrument* is selected and click the next button in the lower right corner.
12. On page 2 of the *Start Measurement Wizard*, choose the 9700 or Veriti instrument you have the Driftcon System set up on from the menu, then click on the *Next* button.
13. Arrow forward through page 3 and visually ensure that the selected protocol is “Driftcon (FAST)” on page 4
14. Continue clicking *Next* through the remaining screens until page 9 when the ***Next*** button changes to ***Finish***. **DO NOT CLICK ON THE FINISH BUTTON BEFORE RUNNING THE PROGRAM ON THE 9700 or Veriti.**
15. For the 9700, press “Run” (F1), then “Start” (F1). For the Veriti, press “Run” from the touch screen. Temperature readings will be collected at 30°C, 95°C, 90°C, 50°C, 70°C, and 60°C.
16. Verify that the reaction volume is set at 20µL, then press “Start” (F1).
17. When the time begins counting down for the first step on the 9700 or Veriti (i.e. when the time changes from 1:00 to 0:59, under the 30.0 setting), click the “***Finish***” button on the Driftcon software to initialize the data collection program. This synchronizes the collection of the software with the running of the 9700 or Veriti.
18. Once the 9700 or Veriti has run through one cycle of temperatures, the Driftcon system will have completed its data collection and the second cycle on the 9700 or Veriti can be aborted.
19. Upon completion of the test, the *Analysis Results* window opens to view the results. Click on *each* temperature on the left side of the window to view the results for each setting.
20. Verify that all temperature settings are highlighted in green, indicating that all tests have passed. A temperature highlighted in red needs to be examined further. If a set-point temperature is highlighted in red, the average deviation from the set-point temperature should not exceed 1.0°C. If the average deviation exceeds 1.0°C then the Driftcon System shall be

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run again. If the temperature setting fails again, a “NOT IN USE” sign shall be placed on the thermal cycler and the supervisor notified.

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Appendix E

7500 Real-Time PCR Instrument Maintenance

The maintenance schedule below has been implemented for use with the HID Real-Time PCR Analysis software using Quantifiler Trio on the 7500 Real-Time PCR Instrument.

Bulb Maintenance:

The halogen bulb should be replaced as needed. After bulb replacement, a background run shall be performed.

Fuse Maintenance:

This procedure is to be performed as needed. The fuse may need to be replaced if the bulb has been changed and the bulb still does not work.

Monthly Maintenance:

The monthly maintenance for the Real-Time PCR instruments consists of the following:

1. Perform a Background Run
2. Quick System Test
3. Thermal Cycler Maintenance
4. System Hardware Function Test

Six (6) Months Maintenance (Calibrations):

The calibration maintenance for the Real-Time PCR instruments consists of the following:

1. Background
2. ROI Calibration
3. Optical Calibration
4. Dye Calibrations (VIC, FAM, ABY, JUN, Mustang Purple)

Directions for background, ROI, optical, and dye calibrations can be found under **Instrument> Instrument Maintenance Manager** in the HID Real-Time PCR Analysis v1.2 software. The six (6) months maintenance may be performed by an Applied Biosystems field engineer as part of the annual maintenance below. An optional RNase P plate may be run if troubleshooting an issue.

Annual (or as needed) Maintenance:

Annual (or as needed) maintenance will be performed by an Applied Biosystems field engineer. Following the maintenance, a performance check must be performed. The performance check will consist of the monthly maintenance procedures and the temperature verification system check.

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General Maintenance Procedures

Monitoring Lamp Status (Periodically):

1. Select **Instrument** then **Lamp Status/Replacement**.
2. The Condition: field indicates one of the following:
 - a. **Good** – The lamp is functioning well. There is no need to replace the lamp bulb at this time. Click **Close**.
 - b. **Failed** – The lamp bulb must be replaced. Click **Close**, then replace the lamp as explained below.
 - c. **Change Soon** – The lamp bulb usage is above 2000 hours. It is recommended to replace the lamp soon. Click **Close**, and replace the lamp.

IMPORTANT! Do not touch the lamp without powder-free gloves. Finger prints shorten the lamp life.

Replacing the lamp (7500):

1. Power off, then unplug the 7500 and allow the instrument to cool for 15 minutes.
2. Open the access door
 - a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
 - b. Open the access door.
3. Remove the lamp from the instrument:
 - a. Slide the lamp release lever downward.
 - b. Firmly grasp the lamp and lift it up and out of the slotted mount.
4. Replace the lamp:
 - a. Slide the lamp release lever downward.
 - b. Firmly grasp the lamp and push in and down into the slotted mount.
5. Make sure the lamp turns on.
 - a. If the lamp does not turn on there may be a problem with the fuse.
 - b. If the lamp turns on, close the access door.
6. Perform a background run.

Replacing the Fuse:

This procedure requires 12.5A, 5 X 20 MM fuses and gloves.

1. Turn off the instrument and unplug it.
2. Remove and inspect the fuse.
3. If the fuse is blown, replace it with the type fuse listed above.
4. Replace the fuse holder into the instrument.
5. Plug the instrument back in. Check to see if the bulb turns back on.



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Monthly Maintenance Procedures

Performing a background run:

This procedure requires a 96-well *Background plate* (this plate is located in the ABI Prism Spectral Calibration Kit) or a 96-well tray with 50µl of deionized water and gloves.

1. Immediately before using the plate, remove it from the freezer, remove the foil packaging, and allow the plate to thaw.
2. Centrifuge the plate to remove all bubbles and force the liquid to the bottom of the tube.
3. Place the plate on the instrument, close the door, and turn the instrument on.
4. Launch the ABI Prism 7500 software.
5. Go to **Instrument > Instrument Manager** and select the **Background** tab. In the Background screen, click **Start Calibration** and follow the on screen instructions for running the background.
6. Analyze the background calibration.
7. If the calibration passes, save the results. If the calibration fails, repeat the calibration or notify the supervisor for course of action.

Performing the Quick System Tests:

This procedure checks the Thermal Cycler well for contamination and also verifies the operation of the shutter and lamp.

1. To check the Thermal Cycler for well contamination: Turn on the 7500 and launch the **ABI Prism 7500 Software**.
2. Make sure that there is no reaction plate in the sample block.
3. Close the instrument door. Select **Instrument > Instrument Manager. Select the ROI Wizard. Select Start Manual Calibration.**
4. Select the FAM filter position (**Filter A**), scroll up to **2048ms**, in the **exposure time (ms)** box. Click **Snapshot**.
5. Observe the fluorescence in the 96-wells, and note wells that have significant fluorescence (this indicates contamination). If wells look fine Click **Done**. Clean the wells as instructed in the Thermal Cycler Maintenance Procedures. Repeat steps 1-5 once wells have been cleaned.
6. To determine acceptable background fluorescence level: Place a new, clean, empty reaction plate without a cover on the sample block.
7. Close the instrument door, take another image at 2048ms.
8. If any wells have significant fluorescence, clean the wells as instructed in the Thermal Cycler Maintenance Procedures. Repeat steps 1-8 of this procedure once the wells have been cleaned.

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Performing Thermal Cycler Maintenance:

This procedure is used to clean wells that show high fluorescence readings. This procedure requires cotton swabs, deionized water, 95% ethanol, and gloves.

Procedure for the 7500:

1. Remove the plate and tray holder.
2. Close the tray.
3. Manually raise the tray from the ROI Inspector Window: Select **File, New**. In the New Document Wizard Click **Finish**. In the software, select **Instrument > Instrument Maintenance Manager**. In the ROI Tab of the Instrument Maintenance Manager select **Start Manual Calibration**.
4. In the ROI Inspector dialog box, click **Move Block**.
5. When the ROI Inspector dialog box displays "Block Down", click **Done**.
6. Power off and unplug the 7500 and allow cooling for 15 minutes.
7. Open the access door to the 7500 by inserting a thin screwdriver into the keyhole on the edge of the access door. Then push to unlatch and open the door.
8. Lift the latch then push the heated cover door to the back of the instrument.
9. Put a small amount of deionized water on a swab and scrub the inside of the wells that exhibit high fluorescence readings.
10. Absorb any excess water with swabs or lint free cloth.
11. Put a small amount of 95% ethanol on a swab and scrub the inside of the wells that exhibit high fluorescence readings.
12. Absorb any excess ethanol with swabs or lint free cloths.
13. Pull the heated cover door to the front of the instrument. Lift the hatch, then secure the heated cover door to the cross bar.
14. Plug in, then power on the 7500.
15. Perform a Background Run or Quick System Tests to determine if further cleaning is needed.

Performing the System Hardware Test (Function Tests):

The purpose of this test is to ensure that the instrument is functioning properly.

1. Turn on the instrument then launch the **7500 Software**.
2. From the toolbar select **Instrument > Function-Test**.
3. Components can be checked individually or as a group. Test all components monthly.
4. To test a component individually, select an item from the left column. The results will be shown as **Pass** or **Fail**.
5. To test all components at once, select the **All Tests** button.
6. If all components **Pass**, select **OK** to close the window.
7. If a component fails, a service engineer may be required. Inform the supervisor and place a note on the instrument letting others know that the instrument is not in use.

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Note: If any of the maintenance procedures fails, inform the supervisor and place a “NOT IN USE” sign on the instrument. A service engineer may be required to resolve the problem.

Driftcon Temperature Verification System:

Note: The Driftcon Verification System is not part of the monthly maintenance for a 7500 Real-time PCR instrument. The Driftcon is used for annual and after service/repair performance checks.

1. Turn on the 7500 Real-time PCR instrument and open the HID Real-Time PCR Analysis software.
2. Login as TBI.
3. Select **Open** and navigate to the appropriate folder (e.g. Driftcon) then to the appropriate 7500 performance check template (e.g. Driftcon (HID).edt and select **Open**.
4. Select **Save As** and save the file as a .eds file in the Driftcon folder (e.g. “Driftcon 3-14-16.eds”).
5. Remove the Driftcon probe from the case and place it on the 96-well 7500 block.
6. Place the thin cork cover on top of the probe.
7. Connect one end of the data cable to the Driftcon Probe and the other end to the hardware module.
8. Connect the USB cable to the back of the hardware module and to the computer. You should see a green light begin blinking on the hardware module.
9. Double click on the Driftcon shortcut icon on the computer to launch the software.
10. Click on the profile icon (e.g. Mr A Admin) and type in the password *driftcon*. If you have a user profile established, log in under that profile.
11. Click on the blue “*Start*” icon to begin the *Start Measurement Wizard*.
12. On page 1, visually check that (q) *PCR* is selected and click the next button in the lower right corner.
13. On page 2 of the *Start Measurement Wizard*, choose the instrument you have the Driftcon System set up on from the menu, and then click on the *Next* button.
14. Arrow forward through page 3 and visually ensure that the selected protocol is “Driftcon (FAST)” on page 4.
15. Continue clicking “**Next**” through the remaining screens until page 9 when the “**Next**” button changes to “**Finish**”. **Note: DO NOT CLICK ON THE FINISH BUTTON BEFORE RUNNING THE PROGRAM ON THE 7500.**
16. On the HID software, click on the **Run** menu tab and select the “**Temperature Plot**” window. Note: Check that the Run Method is set to collect temperatures for 95°C, 30°C, 90°C, 50°C, 70°C, and 60°C.
17. On the HID software, click the green **Start Run** button.

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18. The run status will change from *Not Started* to *Running*. When you see Estimated Time Remaining: 10 min 0 sec appear to the right of the “*Stop Run*” button, click the **Finish** button on the Driftcon software to initialize the data collection program. This synchronizes the collection of the software with the running of the 7500.
19. Upon completion of the test (approximately 10 minutes), the *Analysis Results* window opens to view the results. Click on *each* temperature on the left side of the window to view the results for each setting.
20. Verify that all temperature settings are highlighted in green, indicating that all tests have passed. Temperatures highlighted in red for any of the temperature settings need to be examined further. If a set-point temperature is shown in red, the average deviation from the set-point temperature should not exceed 1.0°C. If the average deviation exceeds 1.0°C then the Driftcon System should be run again. If the temperature setting fails again, a “NOT IN USE” sign shall be placed on the instrument and the supervisor notified.



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Appendix F

**Annual Maintenance and Performance Check for the
Qiagen EZ1 DNA Investigator Kit and BioRobot EZ1 workstation**

BioRobot EZ1 workstation serial # _____

Scientist: _____ **Date(s):** _____

The main objective of this maintenance and performance check is to:

Establish that the use of the Qiagen EZ1 DNA Investigator kit, used in conjunction with the Qiagen BioRobot EZ1 platform, conforms to the specifications of the manufacturer for the extraction of high-quality DNA from forensically relevant samples.

Performance Check Considerations:

Through this performance check the following will be achieved:

1. An assessment of the instrument's temperature and pipetting accuracy.
2. An assessment of the kit and instrument to obtain reliable results.

Studies:

1. *Temperature and pipetting accuracy of the BioRobot EZ1 workstation.*

Objective: Establish that the BioRobot EZ1 workstation is operating according to manufacturer's specifications with regard to temperature and pipetting accuracy.

This study will determine if the temperature of the BioRobot EZ1 workstation is operating within the manufacturer's defined specifications of $\pm 3^{\circ}\text{C}$. Also, this study will evaluate the pipetting accuracy of the BioRobot EZ1 workstation. According to the manufacturer's specifications, pipetting accuracy should be within $\pm 8\mu\text{L}$ for the 100 μL elution volume (92-108 μL) and $\pm 40\mu\text{L}$ for the 500 μL elution volume (460-540 μL).

Test parameters:



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-Temperature accuracy:

Run the temperature accuracy test on the BioRobot EZ1 using the EZ1 card according to the supplemental Qiagen protocol for evaluating temperature accuracy. Set the test temperature to 60°C. Using a NIST traceable thermometer, measure the temperature of the workstation five independent times, allowing the workstation to cool to room temperature between measurements.

-Pipetting accuracy:

Run the 100µL and 500µL protocols on the EZ1 Test Card according to the supplemental Qiagen protocol for evaluating pipetting accuracy. Using a calibrated balance, determine the weight of eluted water. Establish the elution volume for 6 samples (1mg=1µL water).

-For both instrumental parameters, determine if any detectable deviation from the manufacturer's specifications exists.

2. Reliability of kit and instruments

Objective: Establish that the kit and instrument can extract high-quality DNA from a sample type and substrate routinely encountered in forensic casework.

This study is to provide data that establishes DNA extracted using the kit and instrument is amenable to downstream STR applications and that the quality of DNA extracted is acquiescent to obtaining exploitable DNA profiles.

Test parameters:

-Extract one NIST traceable blood sample or known proficiency test sample and reagent blank. The sample and blank shall be quantified, amplified and run on a genetic analyzer.

-Assess the quality of DNA extracted, determined by data obtained from the Quantifiler as well as from the Genemapper ID-X data.

*Include Quantifiler data and electropherograms obtained after analyzing data with Genemapper ID-X.



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Results:

1. Temperature and pipetting accuracy of the BioRobot EZ1 workstation.

Temperature accuracy: Date: _____

Table with 4 columns: Tube, Expected Temperature (°C), Measured Temperature (°C), Temperature Difference (°C). Rows 1-5 with expected values of 60.

Pipetting accuracy: Date: _____

Table with 5 columns: Tube, Weight (g) before run, Weight (g) after run, Difference in weight (g), Pipetted volume (µL). Sections for 100µL and 500µL with rows 1-6.

Conclusions:

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QIAGEN supplemental protocol: evaluating temperature accuracy of the BioRobot EZ1 workstation

Adapted for performance check

This protocol is designed to evaluate the temperature accuracy of the BioRobot EZ1 workstation. The protocol heats water to 60°C. Temperature accuracy should be within defined specifications of $\pm 3^{\circ}\text{C}$.

Procedure:

1. Insert any EZ1 Card completely into the EZ1 Card slot of the BioRobot EZ1.
2. Switch on the BioRobot EZ1.
3. Press "2" to display the tests menu.
4. Press "2" to choose the temperature accuracy test. Use the arrow keys to set the temperature to 60°C.
5. Pipet 200 μL water into the testing hole in the heating block. The testing hole is located distal and between slots 3 and 4 of the heating block.
6. Press "START" to start the protocol.
7. Wait 20min for the heating block to heat to 60°C.
8. Measure the temperature of the water in the testing hole in the heating block using a NIST traceable thermometer.
9. Calculate the temperature accuracy. If the temperature is in the range of 57-63°C, then the accuracy is within the defined specifications.
10. Press "ESC".
11. Allow the water in the heating block to return to room temperature.
12. Repeat steps 3-10 for a total of 5 measurements.
13. Close the workstation door and switch off the BioRobot EZ1.

QIAGEN supplemental protocol: evaluating pipetting accuracy of the BioRobot EZ1 workstation using the EZ1 Test Card

This protocol is designed to evaluate the pipetting accuracy of the BioRobot EZ1 workstation. The BioRobot will pipet either 100 μL or 500 μL of water from one set of tubes to another. Pipetting accuracy should be within defined specifications of $\pm 8\mu\text{L}$ for 100 μL , corresponding to pipetted volumes in the range of 92-108 μL and $\pm 40\mu\text{L}$ for 500 μL , corresponding to pipetted volumes in the range of 460-540 μL .

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Procedure:

1. Label and weigh six (6) Qiagen elution (1.5mL) tubes together with the corresponding caps. Record the weight for each.
2. Insert the EZ1 Test Card completely into the EZ1 Card slot of the BioRobot EZ1.
3. Switch on the BioRobot EZ1.
4. Press "START" to display the protocols menu.
5. Press "1" for the 100 μ L Protocol or "2" for the 500 μ L Protocol.
6. Press any key to proceed through the text displayed in the LCD.
7. Remove the caps from the labeled, weighed tubes, and load the opened tubes into the first row.

8. Fill six (6) un-weighed Qiagen Sample tubes (2mL) without caps with approximately 1mL water. Load the filled, opened tubes into the fourth row (back row).
9. Load 6 tip holders, each containing filtered-tips into the second row.
10. Close the workstation door.
11. Press "START" to start the protocol.
 - The automated procedure takes 2 min.
12. When the protocol ends, the display reads "Protocol Finished".
13. Open the workstation door.
14. Press "ESC" to return to the main menu.
15. Remove the labeled tubes from the first row and cap them securely with the corresponding weighed caps.
16. Weigh the closed tubes and record the weights.
17. Calculate the difference in weight of each tube by subtracting the weight of the empty tube and cap, recorded in step 1.
18. Calculate the accuracy of pipetting*. If the difference in weight is in the range of 92-108mg for the "100 μ L Protocol" or 460-540mg for the "500 μ L Protocol", then the accuracy is within the defined specifications. If the accuracy is not within the defined specifications, contact QIAGEN Instrument Service to recalibrate the instrument.
19. Clean the EZ1.

*1mg = 1 μ L water

Note: If the above yearly maintenance/calibration is performed by an outside vendor, the EZ1 Biorobot shall be performance checked by the extraction, quantification, amplification, and genotyping of a NIST-traceable or proficiency sample and a reagent blank. Documentation of acceptance will be kept in the appropriate logbook along with the initials of the QC analyst.



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Appendix G

Yearly Maintenance and Performance Check for the Qiagen EZ1 DNA Investigator Kit and BioRobot EZ1-XL workstation

BioRobot EZ1 Advanced XL workstation serial # _____

Scientist: _____ Date(s): _____

The main objective of this maintenance and performance check is to:

Establish that the use of the Qiagen EZ1 DNA Investigator kit, used in conjunction with the Qiagen BioRobot EZ1 Advanced XL platform, conforms to the specifications of the manufacturer for the extraction of high-quality DNA from forensically relevant samples.

Performance Check Considerations:

Through this performance check the following will be achieved:

1. An assessment of the instrument's temperature and pipetting accuracy.
2. An assessment of the kit and instrument to obtain reliable results.

Studies:

1. *Temperature and pipetting accuracy of the BioRobot EZ1 workstation.*

Objective: Establish that the BioRobot EZ1 Advanced XL workstation is operating according to manufacturer's specifications with regard to temperature and pipetting accuracy.

This study will determine if the temperature of the BioRobot EZ1 Advanced XL workstation is operating within the manufacturer's defined specifications of $\pm 3^{\circ}\text{C}$. Also, this study will evaluate the pipetting accuracy of the BioRobot EZ1 Advanced XL workstation. According to the manufacturer's specifications, pipetting accuracy should be within $\pm 8\mu\text{L}$ for the 100 μL elution volume (92-108 μL) and $\pm 40\mu\text{L}$ for the 500 μL elution volume (460-540 μL).

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Test parameters:

-Temperature accuracy:

Run the temperature accuracy test on the BioRobot EZ1 Advanced XL using the EZ1 Advanced XL test card according to the supplemental Qiagen protocol for evaluating temperature accuracy. Set the test temperature to 60°C. Using a NIST traceable thermometer, measure the temperature of the workstation five independent times, allowing the workstation to cool to room temperature between measurements.

-Pipetting accuracy:

Run the 100µL and 500µL protocols on the EZ1 Advanced XL Test Card according to the supplemental Qiagen protocol for evaluating pipetting accuracy. Using a calibrated balance, determine the weight of eluted water. Establish the elution volume for 14 samples (1mg=1µL water).

-For both instrumental parameters, determine if any detectable deviation from the manufacturer's specifications exists.

2. *Reliability of kit and instruments*

Objective: Establish that the kit and instrument can extract high-quality DNA from a sample type and substrate routinely encountered in forensic casework.

This study is to provide data that establishes DNA extracted using the kit and instrument is amenable to downstream STR applications and that the quality of DNA extracted is acquiescent to obtaining exploitable DNA profiles.

Test parameters:

-Extract one NIST traceable blood sample or known proficiency test sample and reagent blank. The sample and blank shall be quantified, amplified and run on a genetic analyzer.

-Assess the quality of DNA extracted, determined by data obtained from the Quantifiler as well as from the Genemapper ID-X data.

*Include Quantifiler data and electropherograms obtained after analyzing data with Genemapper ID-X.

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Results:

1. *Temperature and pipetting accuracy of the BioRobot EZ1 XL workstation.*

Temperature accuracy: Date: _____

Tube	Expected Temperature (°C)	Measured Temperature (°C)	Temperature Difference (°C)
1	60		
2	60		
3	60		
4	60		
5	60		

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Pipetting accuracy: Date: _____

Tube	Weight (g) before run	Weight (g) after run	Difference in weight (g)	Pipetted volume (µL)
100µL				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
500µL				
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

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2. *Reliability of kit and instruments*

Include Quantifiler data and electropherograms obtained after analyzing data with Genemapper ID.

Conclusions:



QIAGEN supplemental protocol: evaluating temperature accuracy of the BioRobot EZ1-XL workstation

This protocol is designed to evaluate the temperature accuracy of the BioRobot EZ1-XL workstation. The protocol heats water to 60°C. Temperature accuracy should be within defined specifications of $\pm 3^{\circ}\text{C}$.

Procedure:

1. Insert any EZ1 Advanced XL Card completely into the card slot of the EZ1 Advanced XL.
2. Switch on the EZ1 Advanced XL. The power switch is located at the left rear of the instrument.
3. Press “3” to display the tests menu.
4. Press “2” to choose the temperature accuracy test. Use the arrow keys to set the temperature to 60°C.
5. Pipet 200 μL water into the testing hole in the heating block.
6. Press “START” to start the protocol.
7. Wait 20 min for the heating block to heat to 60°C.
8. Measure the temperature of the water in the testing hole in the heating block using an appropriate thermometer.
9. Calculate the temperature accuracy. If the temperature is in the range of 57–63°C, then the accuracy is within the defined specifications of $\pm 3^{\circ}\text{C}$.
10. To run another temperature accuracy test, press “ESC” and follow the protocol from step 3. Otherwise close the EZ1 Advanced XL door, press “ESC”, and switch off the EZ1 Advanced XL.

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**QIAGEN supplemental protocol: evaluating pipetting accuracy
of the BioRobot EZ1 workstation using the EZ1 Test Card**

Procedure:

1. Label and weigh fourteen (14) Qiagen elution tubes (1.5 mL) together with the corresponding caps. Record the weight for each set of tube and cap.
2. Insert the EZ1 Advanced XL Test Card completely into the card slot of the EZ1 Advanced XL.
3. Switch on the EZ1 Advanced XL. The power switch is located at the left rear of the instrument.
4. Press “START” to display the protocols menu.
5. Press “1” for the 100 μ L Protocol or “2” for the 500 μ L Protocol.
6. Press any key to proceed through the text shown in the display and start worktable setup. The text summarizes the following steps which describe the loading of the worktable. Wear gloves when loading the required items on the worktable.
7. Remove the caps from the labeled, weighed 1.5 mL tubes, and load the opened tubes into the first row.
8. Fill fourteen (14) Qiagen Sample tubes (2mL) without caps with approximately 1mL water. Load the filled, opened tubes into the fourth row (back row).
9. Load 14 tip holders, each containing filtered-tips into the second row.
10. Close the EZ1 Advanced XL door.
11. Press “START” to start the protocol. The automated procedure takes 2 min.
12. When the protocol ends, the display shows “Protocol finished”. Open the EZ1 Advanced XL door.
13. Press “ESC” to return to the main menu.
14. Remove the labeled tubes from the first row (front row), and cap them securely with the corresponding weighed caps.

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15. Weigh the closed tubes and record the weights.

16. Calculate the difference in weight of each tube by subtracting the weight of the empty tube and cap, recorded in step 1.

17. Calculate the accuracy of pipetting. If the difference in weight is in the range of 92–108 mg for the “100 μ L Protocol” or 460–540 mg for the “500 μ L Protocol”, then the accuracy is within the defined specifications. If the accuracy is not within the defined specifications, contact QIAGEN Instrument Service.

1 mg = 1 μ L water. The weight range of 92–108 mg corresponds to 92–108 μ L water and the weight range of 460–540 mg to 460–540 μ L. Fourteen results will be obtained in total for each EZ1 Advanced XL.

18. To run another pipetting accuracy protocol, carry out step 1 of the protocol, and then follow the protocol from step 4. Otherwise close the EZ1 Advanced XL door, and switch off the EZ1 Advanced XL.

19. Clean the EZ1 Advanced XL

Note:

If the above yearly maintenance/calibration is performed by an outside vendor, the EZ1 Biorobot shall be performance checked by the extraction, quantification, amplification, and genotyping of a NIST-traceable or proficiency sample and reagent blank. Documentation of acceptance will be kept in the appropriate logbook along with the initials of the QC analyst.

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Appendix H

Thermometer Check (Annually)

The minimum requirements of a performance check of a thermometer used for performing performance checks may be accomplished through: (1) certification by an outside vendor; or (2) in-house by the comparison of one or more temperature readings at various time intervals against another NIST-traceable thermometer.

For example, a NIST-traceable thermometer certified for two years and used for conducting performance checks on equipment shall require the annual performance check. A NIST-traceable thermometer certified for two years that is not used for conducting performance checks does not require the annual performance checks and may be used until the certification expires. A NIST-traceable thermometer to be used beyond its certification date shall be recertified or be subject to the annual performance-check requirements.

Fill in below when performance checking a thermometer in-house:

NIST traceable thermometer serial #/ID _____

Thermometer (undergoing performance check) serial #/ID _____

Time Interval	NIST Traceable Temperature	Thermometer Temperature

Pass / Fail _____

Analyst

Date

Thermometers used for performing performance checks shall be discarded or re-certified if reading is $\pm 1^{\circ}\text{C}$ from the NIST traceable thermometer.

Critical thermometers used for dry baths shall be discarded or recertified if reading is $\pm 2^{\circ}\text{C}$ from the NIST traceable thermometer.

Less accurate thermometers used in refrigerators and freezers shall be discarded or re-certified if reading is $\pm 5^{\circ}\text{C}$ from the NIST traceable thermometer.

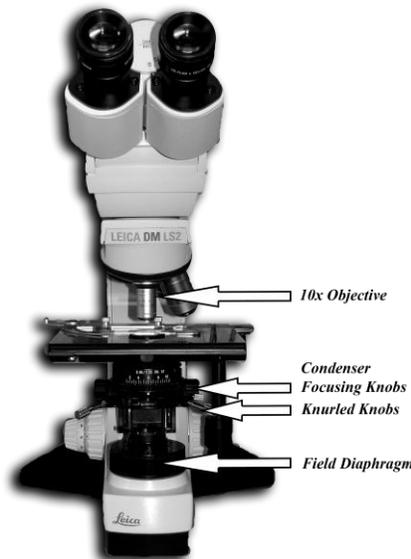


Appendix I

KÖHLER ILLUMINATION

To set up Köhler illumination: Switch on the light source and make sure that light is coming through the field diaphragm at the base of the microscope stand.

1. Place your specimen on the stage and turn the nosepiece (which holds the objective lenses) to the 10X lens. Open the field diaphragm as far as it will go.
2. Now bring your specimen into focus (details are as sharp as they can be) with the coarse and fine focusing knobs. If the light is too bright, reduce it with the rheostat on the light source.
3. When the specimen is in focus, close the field diaphragm all the way. Then, move the condenser up or down with the condenser focusing knobs until the field diaphragm silhouette (shaped like an octagon) is sharply defined and focused properly with clear edges.
4. If the field diaphragm silhouette is not centered within the field of view, adjust it with the two knobs (knurled knobs) coming out diagonally from the condenser.
5. Now open up the field diaphragm until the edge of the diaphragm silhouette is outside the field of view. You should also now be able to turn up the light at the power source.
6. Your specimen should be properly illuminated and should give you a great image. If it does not, check to make sure your lenses and other optical components are clean. If you still cannot get a good image place a “Do Not Use” sign on the microscope and call for repair.



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Appendix J

NIST Standards and NIST Traceable Samples

Each commercially purchased NIST standard will be stored according to the manufacturer's recommendations. If no expiration date is available from the manufacturer, an expiration of one year from the date the NIST standard is received into the laboratory will be used for the expiration date.

NIST traceable blood swatches will be labeled with a unique lot number and stored at room temperature. The swatch will be labeled with the date prepared and will have an expiration date of five years. The documentation and data indicating that the swatch is NIST traceable will be maintained in a notebook. Samples are made NIST-traceable by amplifying and genotyping the sample alongside a purchased NIST standard. Only a sample collected from an individual is made NIST-traceable, not the individual.

NIST traceable extracts will be refrigerated at 5°C or frozen at -20°C. The NIST traceable extract tube will be clearly labeled with the lot number and expiration date. The expiration date will be five years from the time the sample was extracted. The documentation and data indicating that the extract is NIST traceable will be maintained in a notebook along with the concentration of the extract.

The NIST samples, extracts, and swatches shall be handled using the same quality control measures as indicated for casework samples to avoid contamination or degradation.

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Appendix K

On-Site Visit Checklist and Sign Off

Vendor Lab _____

Date(s) of site visit _____

On site visit performed by _____

Laboratory Contact Person _____

Laboratory Technical Leader _____

- | | Yes | No |
|---|--------------------------|--------------------------|
| 1. Is the lab presently performing any analysis for any agency in state where profiles are to be entered in CODIS? | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Was a tour of the facility provided? | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Is the lab designed to provide adequate security and minimize contamination (FBI/QAS 6.1)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. Is access to the lab controlled and limited (FBI/QAS 6.1)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. Does the lab have and follow a documented evidence control system or sample inventory control system for handling and preserving the integrity of physical evidence (FBI/QAS 7.1)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. Is each sample labeled with a unique identifier in accordance with established lab policy (FBI/QAS 7.1.1)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. Does the lab maintain a chain of custody for all evidence (FBI/QAS 7.1.2)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. Is the lab accredited? | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. Was a copy of the current FBI/QAS audit provided for review and TBI records? | <input type="checkbox"/> | <input type="checkbox"/> |
| 10. Are the audits of the lab completed and documented annually (FBI/QAS 15.1)? | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. Were the audits found to be satisfactory? | <input type="checkbox"/> | <input type="checkbox"/> |
| 12. Were corrective action responses satisfactory? | <input type="checkbox"/> | <input type="checkbox"/> |

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- 13. Were copy(ies) of certificates of accreditation provided for TBI records?
- 14. Was the Quality Assurance Program satisfactory?
- 15. Are proficiency tests satisfactory?
- 16. Were case files satisfactory?
- 17. Were the technical requirements for TBI to assume ownership of data addressed?

Notes:

Review and approval:

CODIS Administrator (if applicable) _____ Date _____

Technical Leader _____ Date _____

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Appendix L Quality Control Check of Quantifiler Trio Kit

Date received: _____ Date tested: _____ Analyst's initials: _____
Kit lot #: _____ Expiration date: _____
Reaction Mix lot #: _____ Primer Mix lot #: _____
DNA Standard lot #: _____ Dilution Buffer lot #: _____

Prepare the standards set from the new kit and run on a Real-Time PCR instrument as described in the laboratory protocol.

The resulting standard curve should have an R^2 value greater than 0.98 for the small autosomal target (SA), the Y target (Y), and the large autosomal target (LA). The slope which represents the PCR amplification efficiency for the assay should be between -3.0 and -3.6 for the SA and Y targets and between -3.1 and -3.7 for the LA target. If the R^2 value is less than 0.98 and/or the slope is outside of the specified values, the run must be repeated. If the run fails again, the Supervisor must be notified. The Y-intercept values for each target should also be compared to the Y-intercept values from the previous Quantifiler Trio kit QC check. If the Y-intercept values for the SA or Y target of the new kit varies by more than ± 0.32 Ct from the previous kit QC, the Supervisor should be notified. A difference between the Y-intercept values of greater than ± 0.32 may require the Supervisor to notify users of the new kit about the variation between the new kit and previous kit.

Place documentation in the appropriate QC notebook.

R ² Value:	SA _____	Y _____	LA _____
Slope Value:	SA _____	Y _____	LA _____
Y-Intercept from new kit:	SA _____	Y _____	LA _____
Y-Intercept from previous kit:	SA _____	Y _____	LA _____
Y-Intercept difference:	SA _____	Y _____	LA _____

R² values and slope values are within range for each target: Yes No

Technical reviewer's initial's and date _____ New Kit: PASS FAIL

Corrective action (if kit fails QC check):

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Appendix M

Qiagen EZ1 DNA Investigator Kit Quality Control Check

Technical reviewer's initials and date of approval: _____

New kit lot #: _____

Date Received: _____

Expiration Date: _____

Analyst's Initials: _____

Date kit QC checked: _____

Procedure:

Extract a NIST traceable blood sample or a proficiency test blood sample (with known results) and a reagent blank using the new Qiagen EZ1 DNA Investigator Kit reagent cartridges along with the following reagents, if applicable:

Buffer G2 L/N: _____

Buffer MTL L/N: _____

cRNA L/N: _____

proK L/N: _____

Extract the sample and reagent blank using the nuclear DNA pretreatment protocol. Purify the sample and blank using the EZ1 or EZ1-XL. Quantify and amplify the sample and reagent blank. Run the amplified products on a genetic analyzer. Place documentation in the appropriate QC notebook.

The new kit components shall be marked as passing if the blood sample provides an expected profile of sufficient quality and the reagent blank contains no contamination.

QC Check: PASS FAIL

Corrective Action (if kit fails QC check):

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Appendix N

Quality Control Check of GlobalFiler Kit

Date received: _____ Date tested: _____ Analyst's initials: _____

Kit lot #: _____ Expiration date: _____

Master Mix lot #: _____ Primer Set lot #: _____

Control DNA lot #: _____ Allelic Ladder lot #: _____

Note: The sample chosen to QC the amplification kit must be a NIST traceable sample or a proficiency test sample with known results.

	Expected:	Results:
D3S1358		
vWA		
D16S539		
CSF1PO		
TPOX		
Y-Indel		
AMEL		
D8S1179		
D21S11		
D18S51		
DYS391		
D2S441		
D19S433		
TH01		
FGA		
D22S1045		
D5S818		
D13S317		
D7S820		
SE33		
D10S1248		
D1S1656		
D12S391		
D2S1338		

Controls and QC sample are free of contamination: Yes No

Positive Control and QC sample provided correct profiles: Yes No

Technical Reviewer's initials and date: _____ New Kit: PASS FAIL

Corrective Action (If QC of kit fails): _____



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Appendix O

3500 Monthly Maintenance Instructions

1. Back-Up: Use the following pathway starting at the desktop to access sample files for back-up of the current month's runs: My Computer>D drive>Applied Biosystems>3500>Data.
 - a. Right click and create a folder labeled with the month and year of the runs (e.g. January 2014)
 - b. Click and drag all runs for this month into the folder
 - c. Save the folder to two sources. A USB and an external hard drive are recommended. If a USB or external hard drive is not available, a CD/DVD may also be used to back-up the run folder. USB's should be labeled with the instrument name and year of runs. A CD/DVD should be labeled with the instrument name and month/year of runs. It is recommended to place only one month of runs on a CD and one year of runs on a USB.
2. Archive: It is recommended to keep only three months of runs on the 3500 hard drive (e.g. after April 30 the only runs on the hard drive should be February/March/April). Previous runs should be removed from the 3500 computer.
 - a. To archive a month of runs, the run folder from the D drive should be dragged to the recycle bin. The recycle bin should then be emptied.
3. Purge plates from the Library
 - a. From the Library, select Plates. Highlight the plates from the month to purge and select delete.
4. Run the Wash Pump and Channels wizard.
 - a. From the Maintenance Wizards screen, click Wash Pump and Channels. Follow the prompts in the Wash Wizard window.
5. Flush the water trap (pump trap)
 - a. Follow the directions found on page 231 of the Applied Biosystems 3500 / 3500xL Genetic Analyzer Revision dated May 2012.

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6. Defragment the C and D drives

- a. Go to the Start>All Programs>Accessories>SystemTools>Disk Defragmenter. Follow the prompts to defragment the C and D drives.
- b. Restart the computer and instrument.



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Appendix P

Quality Control Check of DNA Reagents

Date tested: _____

Analyst's initials: _____

	Date made	Lot #	Expiration
Stain Extraction Buffer:	_____	N/A	_____
0.39M DTT:	_____	_____	_____
Proteinase K:	_____	_____	_____
P:C:I:	_____	_____	_____
Non-sperm Extraction Buffer:	_____	N/A	_____
Sperm Extraction Buffer:	_____	N/A	_____
Sperm Wash Buffer:	_____	N/A	_____
TE Buffer:	_____	N/A	_____

	Expected			Results		
	Known:	Non-sperm:	Sperm:	Known:	Non-sperm:	Sperm:
D3S1358						
vWA						
D16S539						
CSF1PO						
TPOX						
Y-Indel						
AMEL						
D8S1179						
D21S11						
D18S51						
DYS391						
D2S441						
D19S433						
TH01						
FGA						
D22S1045						
D5S818						
D13S317						
D7S820						
SE33						
D10S1248						
D1S1656						
D12S391						
D2S1338						

All associated blanks were free of contamination:
 Technical Reviewer's initials and date: _____

DNA reagents: YES NO
 PASS FAIL

Corrective Action (If QC of reagents fail): _____

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Appendix Q Quality Control Check of Yfiler Plus Kit

Date received: _____ Date tested: _____ Analyst's initials: _____
 Kit lot #: _____ Expiration date: _____
 Master Mix lot #: _____ Primer Set lot #: _____
 Control DNA lot #: _____ Allelic Ladder lot #: _____

Note: The sample chosen to QC the amplification kit must be a NIST traceable sample or a proficiency test sample with known results.

	Expected:		Results:	
DYS576				
DYS389I				
DYS635				
DYS389II				
DYS627				
DYS460				
DYS458				
DYS19				
YGATAH4				
DYS448				
DYS391				
DYS456				
DYS390				
DYS438				
DYS392				
DYS518				
DYS570				
DYS437				
DYS385				
DYS449				
DYS393				
DYS439				
DYS481				
DYF387S1				
DYS533				

Controls and QC sample are free of contamination: Yes No
 Positive Control and QC sample provided correct profiles: Yes No
 Technical Reviewer's initials and date: _____ New Kit: PASS FAIL
 Corrective Action (If QC of kit fails): _____

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Appendix R

EZ1 / EZ1 XL Performance Check After Repair/Service

Analyst: _____

Instrument Serial Number: _____

Date Repair/Service Completed: _____

Date Instrument Tested: _____

	NIST / PT Result		Reagent Blank Result		NIST / PT Expected
D3S1358					
vWA					
D16S539					
CSF1PO					
TPOX					
Y-Indel					
AMEL					
D8S1179					
D21S11					
D18S51					
DYS391					
D2S441					
D19S433					
TH01					
FGA					
D22S1045					
D5S818					
D13S317					
D7S820					
SE33					
D10S1248					
D1S1656					
D12S391					
D2S1338					

The EZ1 or EZ1 XL performance check shall be marked as passing if the NIST or PT sample result provides the expected profile with no contamination in the reagent blank or sample.

Technical Reviewer's initials and date: _____

PASS FAIL

Corrective Action (If performance check fails): _____



Appendix S

Reinterpretation of Data Typed with Legacy Amplification Kits

- 1.1 DNA typing results previously generated with Profiler Plus/Cofiler or Identifiler Plus are referred to as legacy data. These amplification kits are no longer in use and analysts are no longer proficiency tested on these kits. In order to reinterpret legacy data, analysts and technical reviewers will be required to complete training in the legacy kit requiring reinterpretation.
- 1.2 The following are examples of situations that are considered reinterpretation:
 - 1.2.1 A comparison requiring analysis/interpretation of an electropherogram generated from a legacy amplification kit e.g. assessing/evaluating allele calls, genotype calls, assessing potential dropout.
 - 1.2.2 A change in the assumption used for interpretation of legacy data.
 - 1.2.3 Adding or removing alleles or loci from statistical calculations.
- 1.3 Comparisons to single source profiles and to DNA profiles with previously documented genotypes are not considered reinterpretation.
- 1.4 Analysts or technical reviewers who have been previously qualified in a legacy amplification kit for which they no longer proficiency test will be required to maintain or reestablish their technical skills and knowledge prior to reinterpretation of legacy data. The technical leader will authorize the analyst or technical reviewer to reinterpret legacy data for no more than a two-year period before requiring reestablishment of technical skills and knowledge. In order to reinterpret legacy data, the following training will be required for an analyst or technical reviewer who has been previously qualified in a legacy kit:
 - 1.4.1 Review of the Profiler Plus/Cofiler and/or Identifiler Plus validations.
 - 1.4.1.1 The Profiler Plus/Cofiler validation review will include validations from both the AB 310 and AB 3130 genetic analyzers.
 - 1.4.1.2 The Identifiler Plus validation review will include validations from both the AB 3130 and AB 3500 genetic analyzers.

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- 1.4.2 Review of the Profiler Plus/Cofiler and/or Identifiler Plus standard operating procedures.
 - 1.4.2.1 For Profiler Plus/Cofiler data generated from both AB 310 and AB 3130 genetic analyzers, an analyst will refer to Revision 9 of the STR Procedure Manual for information and interpretation guidelines.
 - 1.4.2.2 For Identifiler Plus data generated from an AB 3130 genetic analyzer, an analyst will refer to Revision 3 of the Forensic Biology STR Typing Manual.
 - 1.4.2.3 For Identifiler Plus data generated from an AB 3500 genetic analyzer, an analyst will refer to Revision 6 of the Forensic Biology STR Typing Manual.

- 1.5 For an analyst or technical reviewer to be qualified in reinterpretation of legacy data for which they were not previously qualified in the laboratory, the analyst will need to complete the following training in order to demonstrate the technical skills and knowledge required to interpret data, reach conclusions, and generate reports.
 - 1.5.1 Review the Profiler Plus/Cofiler and/or Identifiler Plus validations.
 - 1.5.1.1 The Profiler Plus/Cofiler validation review will include validations from both the AB 310 and AB 3130 genetic analyzers.
 - 1.5.1.2 The Identifiler Plus validation review will include validations from both the AB 3130 and AB 3500 genetic analyzers.
 - 1.5.2 Review the Profiler Plus/Cofiler and/or Identifiler Plus standard operating procedures.
 - 1.5.2.1 For Profiler Plus/Cofiler data generated from both AB 310 and AB 3130 genetic analyzers, an analyst will refer to Revision 9 of the STR Procedure Manual for information and interpretation guidelines.
 - 1.5.2.2 For Identifiler Plus data generated from an AB 3130 genetic analyzer, an analyst will refer to Revision 3 of the Forensic Biology STR Typing Manual.



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- 1.5.2.3 For Identifiler Plus data generated from an AB 3500 genetic analyzer, an analyst will refer to Revision 6 of the Forensic Biology STR Typing Manual.
- 1.5.3 Training with an analyst who was previously qualified in the legacy kit. The training should include the documented review and reinterpretation of previous case files and/or proficiency tests. The training will include the proper interpretation, reporting, and statistical assessment of the legacy data. Training for Profiler Plus/Cofiler case files and/or proficiency tests should include data generated from both AB 310 and AB 3130 genetic analyzers. Training for Identifiler Plus case files and/or proficiency tests should include data generated from both AB 3130 and AB 3500 genetic analyzers.
- 1.5.4 At least three previous case files and/or proficiency tests should be included in the training for each legacy kit. For Profiler Plus/Cofiler, at least one case file and/or proficiency test will include AB 3130 genetic analyzer data if the other two files have data generated from an AB 310 genetic analyzer or vice versa. For Identifiler Plus, at least one case file and/or proficiency test will include AB 3130 genetic analyzer data if the other two files have data generated from an AB 3500 genetic analyzer or vice versa.
- 1.5.5 The analyst or technical reviewer must successfully complete a competency test requiring practical applications of interpretation. Successful completion will be reaching correct conclusions and generating accurate report results. It is recommended that electropherograms from a previous proficiency test be used for the competency test(s).
- 1.5.6 The technical leader will review the training and competency testing. The technical leader will document the successful completion of the competency test and authorize the analyst or technical reviewer to reinterpret legacy data. This authorization will be for no longer than a two-year period before requiring reestablishment of technical skills and knowledge.